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EXPERIMENTAL MAMMOGENESIS OF THE RAT AS DETERMINED BY QUANTITATIVE ESTIMATIONS OF NUCLEIC ACIDS AND COENZYMES

by

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I. INTRODUCTION

The role of hormones has been investigated extensively with respect to their effects on mammary gland proliferation and milk production. In spite of continuous, intensive research there appears to be little published concerning the effects of hormonal treatments on the biochemical mechanisms of the mammary gland. Likewise, what hormones are necessary and their optimal synergetic levels for maximal mammary gland growth are not fully known.

This lack of biochemical and endocrinological information is due, in part, to the failure to use acceptable quantitative methods for estimating the degree of mammary gland proliferation.

Much of our present knowledge relating to mammary gland growth has been obtained from qualitative assay methods. While such methods are reasonably accurate for estimating relative growth, they do not in all cases lend themselves as a definite means for studying the biochemistry of the gland. For studying the biochemical-endocrinological interrelations of the gland during growth, lactogenesis and involution quantitative procedures are imperative. Thus, we seek accurate quantitative measurement procedures for studying the mammary gland.

To begin looking for a quantitative criterion, we may ask ourselves one question: what measurable substance or substances, either directly or indirectly are influenced by hormones, will offer an accurate indication of the stage of proliferation, lactation or involution? To this, we can answer desoxyribonucleic acid (DNA), ribonucleic acid (RNA), oxidized diphosphopyridine nucleotide (DPN) and reduced triphosphopyridine

nucleotide (TPN), because of their physiological importance in cell function.

The DNA content of the individual cellular nucleus has been shown to be constant for somatic cells of a given species. As suggested by Davidson and Leslie (39) and Vendrely (189), the DNA constancy per cell can be used to determine the number of cells within a given tissue and can serve as a reference standard by which growth of a tissue may be expressed. Therefore, by using DNA as an index of mammary gland proliferation, one is able to obtain a quantitative measure of changes in the total number of cells of the mammary gland during various physiological and endocrinological periods.

Recent investigations (197,204) have shown that RNA is essential for the direct control in the synthesis of cellular proteins. Thus, it seems reasonable to assume that a measurement of RNA in the mammary gland would represent an index of functional and structural protein biosynthesis, in both the tubular and alveolar cells, and of milk protein productivity in the alveolar cell.

The presence of DPN or, more important, the value of the DPNH/DPN ratio as indicated by Glock and McLean (67,68) and McLean (119) is directly indicative of cellular oxidation, i.e.,

$$DPN^{+} \xrightarrow{+2H^{+}} DPNH + H^{+} + 2e^{-} \qquad (1)$$

These authors further suggested that the ratio value could be equated as a measuring device of cellular oxidative phosphorylation. The function of oxidative phosphorylation is believed to be concerned with supplying

adenosine triphosphate (ATP) to the cell as a source of energy. Thus, measurement of DPN and DPNH would give information relating to the energy requirements of the mammary gland during stages of physiological development.

The measurement of TPNH (or the TPNH/TPN ratio) as suggested by Glock and McLean (67, 68) offers an accurate estimate of biochemical cell reduction, i.e.,

$$2e^- + TPNH + H^+ \xrightarrow{-2H^+} TPN^+ \qquad (2)$$

This reduction has been suggested as being directly involved in fatty acid biosynthesis, because the rate of lipogenesis is limited by the availability of TPNH (176,200). Thus, measurement of TPNH would offer insight into the formation of adipose tissue and milk fat formation prior to, and during, lactopoiesis.

The objectives of this dissertation are to measure the variability of DNA, RNA, DPN, DPNH, TPN, and TPNH in the mammary gland of the rat by using quantitative procedures, and to investigate biochemical and endocrinological mechanisms of this gland. By measuring the variability of the above mentioned substances in the normal rat during pregnancy and lactation, and in the surgically ablated-hormonally treated rat, we may then gain insight into the effects of hormones on the gland and estimate the optimal levels of various exogenous hormones upon growth and normal function of the gland.

II. LITERATURE REVIEW

The "humoral" concept for growth, development and function of the mammary gland was formulated by the early investigations of Grigoriew (78) and Knauer (96). These investigators observed normal mammary gland development in immature female laboratory animals following ovarian transplantation from mature females. This concept envisages that internal secretions, now known as hormones, directly affected the development of the gland. The later investigations of Steinach (168), Athias (11), Bresslau (20), Moore (137), Turner and Schultze (187), and Gardner (60), using grafted ovarian tissue in male and female animals, confirmed the "humoral" concept. Subsequent work by Laqueur et al. (98), Turner and Schultze (187), Halpern and D'Armour (80)--using estrogens; by Turner and Frank (186), Mixner and Turner (130,131)--using progesterone; by Nelson (146), Astwood et al. (10)--using estrogen and progesterone; by Van Heuverswyn (188), Leonard and Reece (99)--using corticosteroids and estrogens; by Weichert and Boyd (198,199)--using desiccated thyroid tissue, and by many others led to the belief that all hormones are involved, either directly or indirectly, in the development of the mammary gland. Those that directly affect mammary development coordinate physiological changes in the gland with changes in the reproductive system. This group of secretions consists of hormones from the ovaries, placenta, anterior pituitary and possibly the adrenal cortex. A second group of hormones would consist of those that are involved indirectly (or synergetically). These affect the metabolism and/or physiology of the mammary gland in

that they influence the general metabolism of the body. In this second group we usually consider insulin, thyroxine and the glucocorticoids, but other hormones are suspected and are being investigated for possible synergetic effects. A brief summary of the physiological changes in the mammary gland induced by each specific hormones is given below. These summaries shall be restricted to those hormones presently under investigation for the mammary glands of the rat and mouse. However, before considering the effect of varying hormones on mammary gland physiology, it is necessary to discuss normal development of the gland during periods in the life of the female rat.

A. Normal Mammary Gland Development

Mammary gland development and proliferation may be considered as consisting of three major periods:

(1) embryonic,

(2) pre-puberty,

(3) puberty.

1. Embryonic development

The first trace of the mammary glands appears on the 11th day of embryonic life (83). The mammary streak arises from a single cell layer of the ventral ectoderm on either side of the midline and extends from the axilla to the inguinal region forming an obvious line which is elevated above the surrounding tissue. Further proliferation results in the localized concentration of epithelial cells along the mammary line which forms the mammary hillocks or buds. The mammary line connect-

ing the buds gradually disappears leaving the isolated cellular masses. The number of buds corresponds to the number of mammary glands which will be formed in the adult animal. The mammary buds gradually sink into the underlying mesenchyme and becomes surrounded by the basement membrane.

The primary sprout begins to develop about the 18th day of fetal life. The Malpighian layer forms a funnel shape outline and extends into the corium. One primary sprout develops from the proximal end of each bud, undergoes extensive branching and extends into the surround stroma. These extensions are the future interlobar ducts. No lobules are formed up to the time of birth (123,124,125).

2. Pre-pubertal development

At birth, the primary duct consists of a solid tube of cells. The secondary and tertiary ducts are all three-to-four cell layers thick. The tertiary ducts have one-to-three terminal branches (121).

By the 10th day after birth, the epithelium of all ducts is two cell layers thick; the inner layer is composed of cuboidal cells, while the outer layer is irregular in size and shape. At two weeks of age the lumen of the primary and secondary sprouts are open and continuous with each other (122).

From the 5th to the 10th week the mammary ducts slowly increase in size; the ducts are increased in length with many new ducts formed (122). End-buds are present on a large number of terminal ducts. This development is greatly accelerated in the period immediately before puberty at approximately 10 weeks (158). No true alveoli are observed from birth to puberty (121,122,184,187).

3. Pubertal development

The sexually mature animal may have one of four possible conditions during its reproductive period:

a. recurring estrous cycles,

b. pregnancy,

c. lactation, and

d. involution.

a. Recurring estrous cycles Mammary gland development has been observed to be closely related to the stage of the estrous cycle in the female rat (24,173). During proestrus the ductal system is composed of small, slender ducts which may or may not contain small mammary buds. At estrous, the buds may proliferate to varying degrees; additional ones may also appear. By the time corpora lutea have formed, an increase in the complexity of the secondary ducts may be seen (173). This additional development may not be completely lost by the next proestrus period, therefore, during the next estrous cycle the mammary glands may progress to a more advanced stage of development.

b. Pregnancy During pregnancy the mammary gland is characterized by the formation of lobules and alveoli and an increase in the glandular tissue. Lobule formation is evident after 10 days of pregnancy in the rat. The alveoli are spherical and gradually increase in size until the time of parturition. Cell division was thought to cease by the 13th day of pregnancy and the number of cells around the alveoli were believed to be maximum at this time. Further increase in size of the mammary gland was thought to occur from increase in size of both the capillaries and

alveoli of the mammary gland. Vacuolization of the alveoli, which is indicative of secretory activity, is found about the 13th day of pregnancy and reaches a maximum by the 19th day of pregnancy (161).

Jeffers (89) observed that the lumina of the alveoli increase in size during the last half of pregnancy; the average diameter at 13 days was 30.8 microns and by the 20th day had increased to approximately 40.6 microns. In contrast to Roberts (161), Jeffers (89) also reported an increase in the number of cells lining a single alveolus. Alveoli from the 12th to 18th day of pregnancy contained an average of 8.09 cells per alveolus, from the 19th-20th day of pregnancy the average number had increased to 10.1 per alveolus.

The colchciine technique was employed by Reece and Warbritton (157) to estimate mitotic rate of the mammary gland during pregnancy. The percentage of cells undergoing division on the 5th day of pregnancy was 4.6, on the 10th day 17.3, on the 15th day 9.3, and on the 20th day 1.1 percent.

c. Lactation Macroscopic aspects of the mammary gland during lactation remains the same as at the end of pregnancy. The glandular parenchyma consists of closely packed lobules and the ducts and alveoli are extended with secretion. The alveolar cells vary in shape due to either the distension of cells by the accumulation of milk or collapse of the cells which follow milk removal. When the cells are empty, the alveolar epithelium is folded and the cells are so elongated that apical cytoplasm projects into the empty lumen. After removal of milk from the alveoli, secretory material again accumulates, and as more is secreted the cells become stretched and thin (159).

Jeffers (89) reported the mammary gland resembles that characteristic of lactation 12 to 15 hours after parturition, and lactation is established 24 hours after parturition.

d. Involution Forty-eight hours after weaning, the mammary glands have the same general appearance as during lactation. The alveoli are widely distended with secretion and secretory activity is still obvious. By the 3rd day the parenchyma is reduced in proportion to stroma, and the glandular substance occurs in groups of small masses. The alveoli which are distended with secretion are greatly reduced, although some secretory activity is still evident. Only a small number of alveoli are present by the 5th day. The majority of the glandular tissue has been replaced by increased amounts of adipose tissue. By the 9th day after weaning, the mammary glands again resemble those of a normal adult virgin animal (115).

B. The Effect of Hormones and Related Substances on Mammary Gland Proliferation

1. Estrogen

Earliest investigations of the "estrogenic" effect on the mammary gland were those of Laquer and associates (98), Turner and Schultze (187), and Halpern and D'Armour (80). These studies suggested that estrogen directly affected the gland by causing ductal growth without alveolar formation. Later studies by Astwood et al. (10), using estrone, by Lewis and Turner (101), using diethylstibestriol, and by Reece and Bivins (156), Nandi (143), and MacDonald and Reece (113), using estradiol, confirmed the investigations of the earlier workers and, in essence,

equated the effect of estrogenic compounds with ductal growth in the mammary gland. Detailed summaries and reviews of the actions of estrogenic substances are given by Folley and Malpress (57), Mayer and Klein (117), Folley (56), Diczfalusy and Troen (42), and Jacobsohn (88).

2. Progesterone

Progesterone, as a single hormone, has been shown in ovarectomized animals by Turner and Frank (185,186), Astwood and associates (9,10) and Mixner and Turner (128,130) to be incapable of stimulating, except in abnormally large amounts, mammary proliferation.

3. Estrogen and progesterone

The observation that a combination of estrogen and progesterone is necessary to induce additional mammary growth was made by Nelson (146). This work was confirmed by Lyons and McGinty (111), Reece and Bivins (156), Turner and associates (35,36,51,52,94,95,192), Reece (113). Elliott and Turner (51,52) using the spreading factor technique found that a particular ratio of estrogen to progesterone was necessary to obtain lobular-alveolar development comparable to normal late pregnancy. These workers suggested 1 μg of estradiol to 5 mg progesterone or a ratio of 1:5000. Damm and Turner (36) suggested that once the particular ratio was established, additional progesterone, with due regard to the ratio, could be added to approximate more closely the normal pregnant condition. Similar results have been obtained by Cortiss (30), Lyons et al. (109), Nandi (143), Moon et al. (135) and Munford (140). From these and other studies, the optimal ratio of estrogen and progesterone necessary to

simulate mammary development in the rat equivalent to that at the end of a normal pregnancy was considered to be 1:2000 (135). The ratio has been now established as 1:3000 by Ahrén and Jacobsohn (3), Anderson and Turner (6), Damm and Turner (38) and Griffith and Turner (74,75) along with other investigators.

The combined effect of estrogen and progesterone has been shown by Damm et al. (35) to be exclusively related to progesterone. These investigators studied the progesterogenic effect of various derived metabolites, i.e., pregnenolone, 17 α hydroxyprogesterone, andostenedione and testosterone, in combination with estrogen. Considering the estrogen-pregesterone effect as unity, the results were 0.29, 0.26, 0.36, and 0.33 percent, respectively, for the metabolites listed above.

4. Adrenal cortical hornes and steroids

The involvement of adrenal cortical hormones and steroids in mammary gland proliferation was first suggested by Van Heuverswyn, et al. (188) working with desoxycorticosterone acetate (DCA). Since that time much research has been generated to study the effects on the mammary gland of individual cortical hormones, alone and in combination with other non-cortical hormones. Investigations by Gaunt (64,65), Leonard and Reece (99), Smith and Braverman (163), Johnson and associates (90,91), Rivera and Bern (160), and Nandi (143)--using DCA; Jacobsohn (86,87), Lyons et al. (108), Johnson and Meites (91), and Ahren and Jacobsohn (4,5)--using adrenal corticaltropic hormone (ACTH); Talwalker et al. (175), Griffith and Turner (75), and Anderson and Turner (6,7)--using hydro-cortisone (HCA); Nelson et al. (148), Anderson and Turner (7,8)--using

DCA and HCA; and Cowie and Tindal (31)--using aldosterone have shown that adrenal cortical hormones act synergistically with estrogenic compounds and progesterone, in the ablated animal, to aid in mammary gland proliferation, but are primarily responsible for proper mammary development and function during the lobular and lactational phases of the gland (109).

Smith and Braverman (163) conducted extensive research on the action of DCA alone and in combination with estradiol and progesterone on the development of the mammary gland of ovarectomized rats. The authors concluded that DCA alone influences ductal proliferation and in combination with progesterone, it does not affect lobule-alveolar growth. When given with estradiol, DCA is less active than the proliferation induced by estrogen and progesterone.

Anderson and Turner (6) obtained mammary growth in adrenalectomized--ovarectomized animals, as measured by DNA, upon giving E, P, and HCA. The measured growth, however, was less than that obtained from giving E and P with HCA. Giving E, P, and DCA, DCA and HCA alone resulted in no proliferation above the ovarectomized control. These same authors (7,8) obtained proliferation in ovarectomized animals upon giving E, P, and HCA.

5. Thyroxine

The effect of thyroxime upon mammary gland proliferation and function is not completely known. The early studies by Dragstedt et al. (47), Weichert and Boyd (198,199), Nelson and Hickman (149), Leonard and Reece (100), Smithcors and Leonard (165), Mixner and Turner (129), and

Smithcors (164) gave confusing and conflicting results. These were believed due to differences in the source and the purity of hormonal preparations (109). More recent and extensive investigations by Blaxter (16), Chen et al. (23), Lyons et al. (109), Moon and Turner (136), Moon (133), and Randle (153)--to mention only a few--have suggested that thyroxine may be a limiting factor in mammary gland growth when estrogens and progesterone secretions are adequate. Moon (133) indicates that thyroxine influences production and secretion of prolactin.

6. Anterior pituitary hormones

The relationship of hormones from the anterior pituitary in mammary gland proliferation was first investigated by Stricker and Grueter (172). These investigators observed that hypophysectomy in the adult female resulted in the cessation of mammary function and growth. This observation was confirmed by the later work of Corner (29), Selye et al. (162), Nelson (146), Nathanson et al. (144), Gardner (61,62), Gardner and White (63), Hooker and Williams (84) and Lyons (104). The work of the above mentioned investigators made it apparent that after hypophysectomy no exogenous injection (excluding an anterior pituitary extract) would produce normal mammary proliferations. This conclusion, along with more advanced purification methods, stimulated further investigations by Lyons et al. (112), Nelson et al. (145), Williams (201), Trentin and Turner (177), Lyons (104,105,106), Desclin (41), Lyons and associates (34,108,109,112), Jacobsohn (85), Cowie and associates (14,18,19,32,33), Randle (154), Flux (55), Nandi (143), Linzell (103), Bern (15), Damm and Turner (37,38), Talwalker and Meites (174), Moon (132), Chandra and Cole (22), Cole and Hopkins (25,26), Ota et al. (151), Danamur (40),

Griffith and Turner (75), MacDonald and Reece (113), Anderson and Turner (8) and Djojosoebagio and Turner (46) in an attempt to determine the specific hormone from the anterior pituitary and the proper synegrists important in mammary gland proliferation. As a result of the above investigations--except for Trentin and Turner (177)--the specific hormone is generally accepted to be prolactin (mammotropin) and is believed to act synergistically with ovarian hormones to produce normal mammary gland growth. The work of Trentin and Turner (177) suggests a specific mammary-developing hormone, mammogen, which is believed to act directly on the gland due to stimulation of the anterior pituitary by the ovarian hormones. However, thorough chemical examinations as reported by Hays and Steelman (82), Li and Evans (102), Lyons (109), Ray et al. (155), and Gala and Reece (59) have failed to yield such a hormone.

A second hormone of importance in mammary gland proliferation is somatotropin (growth hormone). This hormone is believed to stimulate proliferation by increased ductal branching in the gland (108,109,112, 143). The recent work of Knobil and Hotchkiss (97) suggest that somatotropin may further messenger--RNA production. These authors concluded this from experimentation with hypophysectomized animals with and without hormonal therapy. However, Cowie and Tindal (32), with animals hypophysectomized on the 12th day of pregnancy and injected with prolactin and ACTH from the 20th day of pregnancy through the 10th day of lactation obtained 53 percent normal milk production. Thus, it appears that somatotropin acts only as synergist with prolactin for better enhancement of the effect which prolactin has on the mammary gland (22,26).

C. The Effect of Hormones and Steroids on the Concentrations of DNA, RNA, DPN, DPNH, TPN, and TPNH in the Mammary Gland During Proliferation

The effects of exogenous hormonal injections upon the concentrations of DNA, RNA, DPN, DPNH, TPN, and TPNH are not completely known. Our present knowledge is limited because of the wide range of needed experimentation, and the direction of the present experimentation being done. Because of the wide distribution of possible investigations, and concentrated efforts in one area--measurement of DNA concentration--this section will include brief discussion of the suggested physiological function of each of the involved substances in the mammary gland and a short review of investigators working in this area.

1. DNA

The work of Boivin et al. (17) suggested that, for a given species, the DNA concentration per somatic cell was constant. This work was extended and confirmed by Mirsky and Ris (126,127) and Davidson (39). Davidson (39) suggested that the constant DNA concentration could be used to determine the total number of cells in a given tissue. Turner and associates (6,7,8,21,35,36,37,38,46,73,74,75,76,77,94,95,135,136, 192), Greenbaum and associates (71,72), Wang and Greenbaum (193,194), Moon (134), Cole and associates (22,25,26), Tucker and Reece (178,179, 180,181,182,183), and many others have used this criterion as a quantitative measure of mammary gland growth in normal and ablated rats with and without exogenous hormonal injections. These studies empirically

correlate growth changes with those observed and measured qualitatively by histological investigations. However, while most investigators use this method to measure proliferation, Munford (139,140,141) feels histological procedures are the more accurate and should continue to be used.

2. RNA

That the RNA concentration in the mammary gland was an estimate of protein synthesis in the gland was first suggested by Sternberg (171) and Kirkham and Turner (95). The reports of Zamecnik (204), Wang et al. (195), and Watson (197) seemingly confirm the suggested view.

The use of this measurement for studying the gland has been limited. Only until recently with the work of Greenbaum and associates (71,72) Bailie and Morton (12,13), Wang and Greenbaum (193,194), Chandra and Cole (22), Talwalker and Meites (174), Cole and Hopkins (25,26) Ota et al. (151), Tucker and Reece (178,179,180,181,182,183) and MacDonald and Reece (113,114), have we gained some understanding of changes in the protein synthesis capabilities of the gland during normal physiological periods of the animal. The investigations of Cowie and Tindal (32) and Knobil and Hotchkiss (97) offer insight in changes of proteidogenetic abilities in the hypophysectomized animal with hormonal therapy.

3. DPN and DPNH

Glock and McLean (66,67,68) the first to study these substances in the mammary gland, sought information relating to the energy requirements of the gland during lactation. Further investigations by Abraham

et al. (1,2), Stern and Vennestand (169,170), Nakamoto and Vennestand (142), Jones and Gutfreund (92), and Mason (116) have primarily been concerned with the biochemistry of these nucleotides in the normal animal. Pastan et al. (152), however, investigating the reduced form of this nucleotide in vitro with thyroxine therapy observed no change in the concentration of this substance.

4. TPN and TPNH

The work of Glock and McLean (67) initially led the way for further investigations in an attempt to understand the biochemical mechanics of cellular reduction during lactation. This reduction has been indicated by Tepperman and Tepperman (176), Dumont (48,49), Abraham et al. (1,2), Pastan et al. (152), Mason (116), Dils and Popjak (44), Dils and Clark (43) and Coniglio and Popjak (28) to be directly involved with fatty acid biosynthesis in the gland. The reduced form of this nucleotide was found to slightly increase upon the addition of exogenous thyroxine (152) and to increase during lactation (58).

Harding and Nelson (81) and Eik-Nes (50), studying the adrenals in hypophysectomized rats observed no change in the TPNH/TPN ratio. Eik-Nes (50) concluded that there may exist other routes for TPNH production other than the pentose pathway. Other possible sources for the formation of TPNH are: transhydrogenation of TPN, utilizing the hydrogen of either DPNH (93,167,169,170,190) or estradiol (79), and transphosphorylation of DPN (196). Transphosphorylation was observed in the thyroid following the injection of thyrotropin (53,54,152).

Likewise, thyrotropin stimulates the oxidation of glucose via the pentose pathway in the thyroid (138). Thus all systems are suggested to be involved in TPNH production.

III. METHODS

A. Animals

Normal and surgically ablated primiparous Sprague-Dawley-Rolfsmeyer rats, all initially weighing between 205 and 220 gms., were used in this investigation. All animals were fed and watered ad libitum, were cleaned biweekly, and were housed in cages containing only members of the same operative-treatment group combination.

Ovarectomized (O_X) and/or adrenalectomized (A_X) animals were bilaterally ablated via two incisions at one operation. This was done to minimize the amount of cicatrical tissue, to reduce trauma brought about by anesthesia, and to reduce the mortality rate of the animals. All surgery on hormonally treated animals was performed 7 to 10 days prior to the beginning of the experimental period. Surgery on animals receiving no hormonal treatment was completed 27 to 30 days prior to their sacrifice. Nembutal was used as an anesthetic. Both A_X and A_X-O_X animals received special fluid, containing 0.5 percent NaCl and 0.5 percent glucose, from recovery until sacrificed, regardless of hormonal therapy.

Hypophysectomized (H_X) Sprague-Dawley-Rolfsmeyer rats were purchased from Hormone Assay Laboratories; these animals upon their arrival were O_X and/or A_X as necessary and in accordance with the above mentioned experimental time and surgical restrictions. All hypophysectomized animals, i.e., H_X, $A_X - H_X$, $H_X - O_X$, and $A_X - H_X - O_X$ were maintained on special fluid. For simplicity we shall denote $A_X - H_X - O_X$ by T_X

henceforth.

Normal and surgical ablated animals receiving hormonal therapy were given prescribed daily hormonal injections, subcutaneously, in the middle dorsal region at the base of the neck, after the recovery period of from 7 to 10 days. These injections were continued during the experimental period of 19 days to induce lobule-alveolar development. On experimental day 20, the animals were sacrificed. The daily exogenous doses of the hormones used are as shown in Table 1. Table 1 also gives the symbolic notation used henceforth in the text, along with the specific carrying media or the mode of injection used with each of the various hormones.

Upon sacrifice of the animals all abdomino-inguinal mammary glands were removed, separately for each animal, and frozen for DNA and RNA determinations, as described below. Two small portions of the pectoral mammary glands, ranging from 56 mgms to 250 mgms were removed and homogenized separately for the determinations of oxidized and reduced coenzymes, i.e., DPN, TPN, DPNH, and TPNH. In addition, a third portion of pectoral gland was removed for histological purposes.

B. Determinations

In all cases the analytical methods for the determinations were those used by earlier workers in mammary tissue assays. The method of Ogur and Rosen (150) was used to determine DNA and RNA concentrations. The oxidized and reduced forms of the coenzymes were prepared and determined--with the exception of TPNH--by the method of Glock and McLean (66), as modified by Villee (191). Isocitric dehydrogenase, in

Table 1. Injected exogenous hormonal compounds used during experimental period

Hormonal compound	Symbolic notation	Exogenous dose (per animal per day)	Carrier media or mode of injection
Estrone	E	2 μgms	Corn oil
Progesterone	P	6 mgs	Corn oil
Thyroxine	T	3 γ/100 gms Body weight	Saline
Desoxy-corticosterone	DCA	0.25 mgms	Distilled water-crystalline suspension
Hydrocortisone acetate	HCA	500 μgms	Distilled water-crystalline suspension
Growth hormone	STH	1 mgm	Saline
Prolactin (mammogen)	M	1 mgm	Saline

the presence of isocitric acid and the neutralized acid tissue extract, was used to determine DPN, instead of glucose-7-phosphate dehydrogenase as prescribed (27). All samples were analyzed spectrophotometrically, by use of the Spectronic 505 (U.V.), or colorimetrically, by use of the Spectronic 20 (visable).

In order to determine specific concentrations of coenzymes, the method of differences was employed. That is, since isocitric dehydrogenase reduces both DPN and TPN and alcohol dehydrogenase specifically reduces DPN, the total concentration of TPN, in μM per 100 mg of mammary tissue, was calculated by subtracting the total DPN, in μM as determined by alcohol dehydrogenase, from the combined total of DPN and TPN, in μM. Likewise, since diaphorase (from Clostridium kluyveri) oxidizes both DPNH and TPNH, and diaphorase (from pig heart) oxidizes only DPNH, the total concentration of TPNH, in μM, was calculated from combined total, i.e., DPNH and TPNH, in μM minus the total DPNH, in μM.

All drugs were obtained from the Sigma Chemical Co. (Sigma), except two hormones--growth hormone and prolactin, which were a gift from the National Institutes of Health. Diaphorase (Clostridium kluyveri) which was obtained from General Biochemicals.

Histological preparations were prepared by fixing, staining and cleaning pectoral gland tissue in accordance with the method of Lyons and Johnson (107). Once cleared, they were embedded in paraffin, and sectioned at 50 μ, permanent slides were made.

Complete descriptions of the analytical and histological methods used in this experimentation are given in the Appendix.

IV. RESULTS

The results obtained from the present experimentation are given below. To simplify this presentation, these results will be separated into two groups, in accordance to type of mammogenesis--natural and artificial.

A. Natural Mammogenesis

The individual group mean value and the associated standard error of that mean for each of the measured substances for pregnancy and lactation are given, along with pertinent ratios, in Chart 1. In this table, and henceforth, the following notations will be used for simplicity in discussing measurement periods during pregnancy and lactation.

N_{P_i} = normal pregnant animal at the i^{th} day of gestation.

N_{L_i} = normal lactating animal at the i^{th} day of nursing.

In addition, photographs of the anatomical glandular changes occurring in the gland for the 5th, 10th, 15th, and 20th day of pregnancy, and the 1st, 5th, 10th, 15th, and 20th day of lactation are illustrated in Figures 1 through 9. The glands of the normal control ($^{N}P_{o}$) are shown in Figure 10.

B. Artificial Mammogenesis

The results for artificial mammogenesis will be presented in regards to operative-treatment group combinations. However, before

Chart 1. Means and standard errors of means for pregnancy and lactation

Time of measurement	No. exptl. animals	(1) Pertinent statistic	Final animal weight (gms.)	(2) T_{RNA}/100 gms. B.W. (mgm.)	(2) T_{DNA}/100 gms. B.W. (mgm.)	Ratio $\frac{T_{RNA}}{T_{DNA}}$	(3) TPN/100 mgm. M.T. (μM)	(3) TPNH/100 mgm. M.T. (μM)	Ratio $\frac{TPNH}{TPN}$	(3) DPN/100 mgm.M.T. (μM)	(3) DPNH/100 mgm. M.T. (μM)	Ratio $\frac{DPN}{DPNH}$
$N_{P_{20}}$	9	$\bar{x}$	276.9[a]	6.16	7.90	0.78	406.1	678.2	1.67	269.1	7.3	36.86
		$s_{\bar{x}}$		±0.46	±0.38		±15.69	±22.98		±16.89	±0.76	
$N_{P_{15}}$	7	$\bar{x}$	266.0[a]	3.38	6.62	0.51	24.6	298.4	12.13	496.9	8.3	59.87
		$s_{\bar{x}}$		0.23	0.25		3.37	10.91		12.12	0.26	
$N_{P_{10}}$	8	$\bar{x}$	262.2[a]	1.86	4.32	0.43	80.6	851.1	10.56	387.4	4.5	86.09
		$s_{\bar{x}}$		0.27	0.31		8.05	36.69		9.11	0.35	
N_{P_5}	6	$\bar{x}$	228.7	1.68	3.42	0.49	277.1	559.7	2.02	51.1	6.1	8.38
		$s_{\bar{x}}$		0.16	0.20		17.99	26.73		5.01	0.83	
$N_{L_{20}}$	6	$\bar{x}$	227.2[b]	31.17	11.07	2.81	135.9	1700.1	12.51	154.1	16.8	9.17
		$s_{\bar{x}}$		0.62	0.23		8.63	33.08		11.13	0.30	
$N_{L_{15}}$	7	$\bar{x}$	262.9	26.80	10.43	2.57	247.4	5851.0	23.65	292.0	12.8	22.81
		$s_{\bar{x}}$		0.91	0.19		13.92	102.17		19.03	1.75	
$N_{L_{10}}$	6	$\bar{x}$	255.1	23.67	10.59	2.24	104.8	2002.7	19.11	397.4	4.2	92.24
		$s_{\bar{x}}$		0.47	0.21		14.37	74.57		24.48	0.27	
N_{L_5}	8	$\bar{x}$	256.4	16.23	10.47	1.54	76.4	1146.0	15.00	359.2	11.1	32.36
				0.36	0.27		6.07	41.36		22.51	0.96	
N_{L_1}	6	$\bar{x}$	248.4	10.28	8.49	1.21	43.6	557.6	12.79	285.7	2.9	98.52
		$s_{\bar{x}}$		0.29	0.31		3.98	16.45		21.17	0.09	
N_{P_0}	12	$\bar{x}$	226.9	1.12	3.20	0.35	525.8	373.3	0.71	0.0	24.6	0.0
		$s_{\bar{x}}$		0.21	0.29		16.09	14.18		0.0	4.26	

Notes used in this chart and Charts 2, 3, 4, 5, and 6:

(1) $\bar{x}$ = Sum of individual observations for a given measurement period divided by the total number of observations for that period, i.e.,

$$\bar{x} = \sum_{i=1}^{n} x_i/n, \text{ and}$$

$$s_{\bar{x}} = \left[\frac{2(x_i-\bar{x})^2}{n(n-1)} \right]^{1/2} = \frac{s}{\sqrt{n}}, \text{ where } s \text{ is the estimated standard error of the population for a given measurement period.}$$

(2) T_{RNA}/100 gms. B.W. is total RNA (or DNA) per 100 gms. body weight of animal, in mgms.

(3) TPN/100 mgm. M.T. is total TPN (TPNH, DPN or DPNH) per 100 mgms. of wet weight of mammary gland tissue, in μM.

[a] Final animal weight adjusted for fetal weight.

[b] Mother was still nursing young.

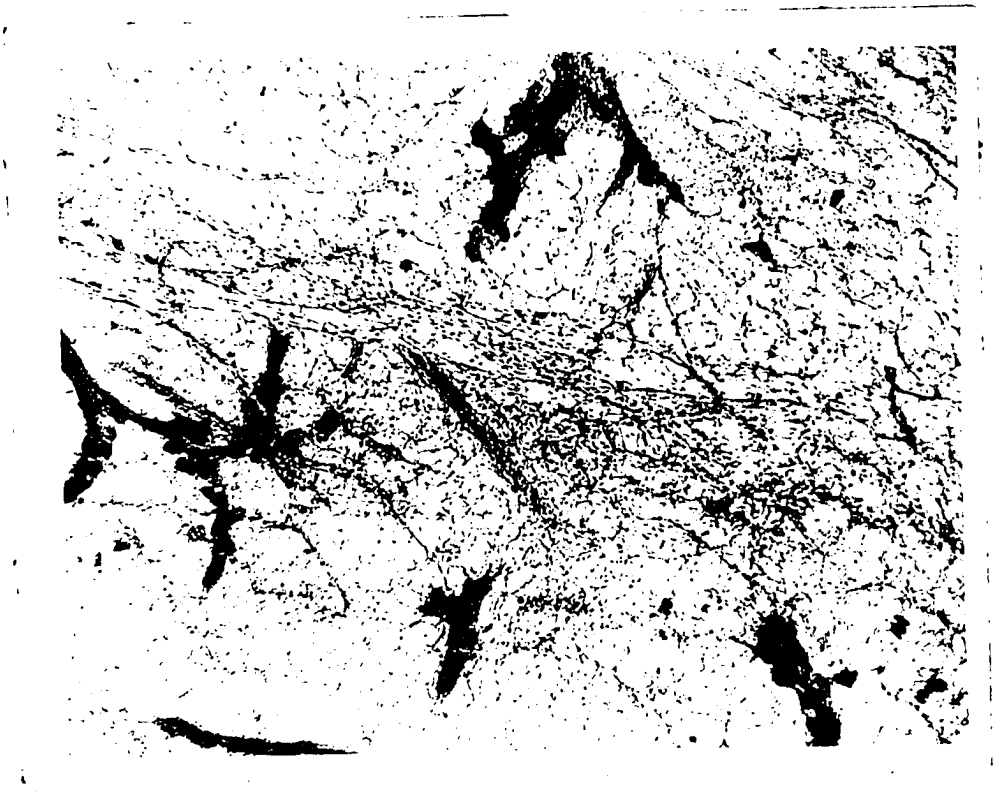

Figure 1. Mammary gland from a normal pregnant (N_{P_5}) rat

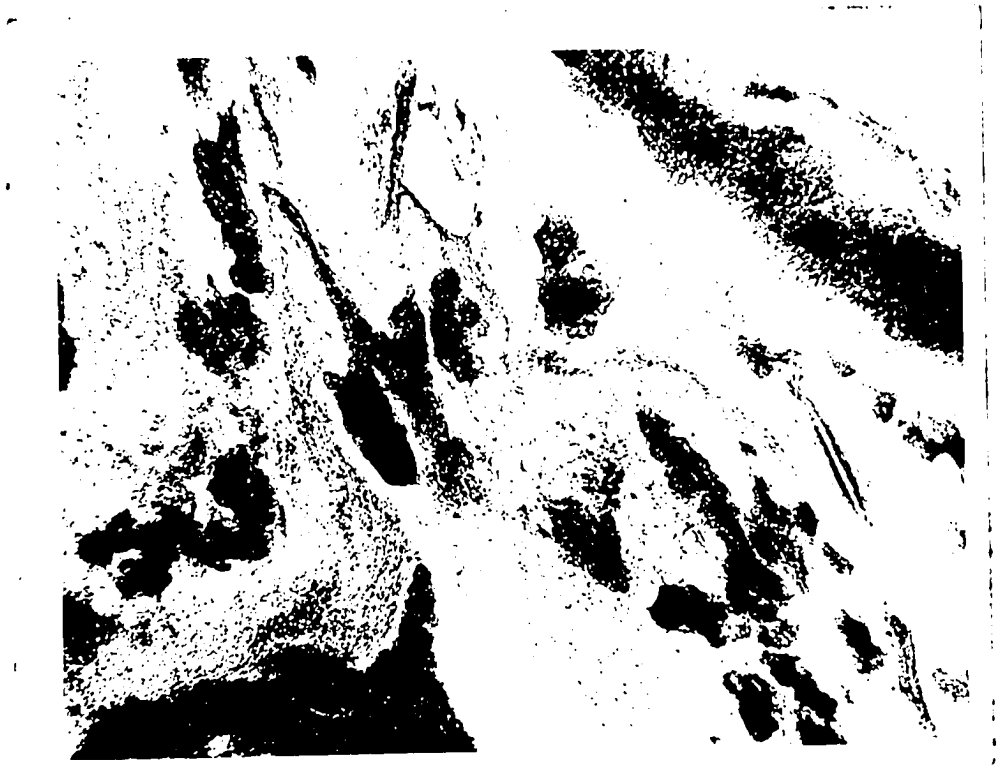

Figure 2. Mammary gland from a normal pregnant ($N_{P_{10}}$) rat

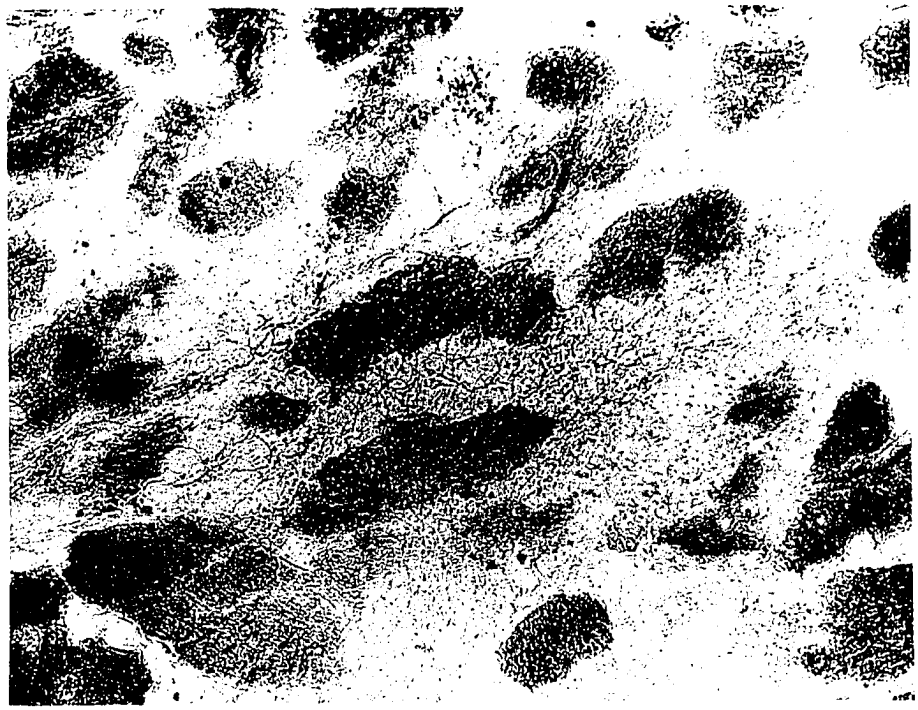

Figure 3. Mammary gland from a normal pregnant ($N_{P_{15}}$) rat

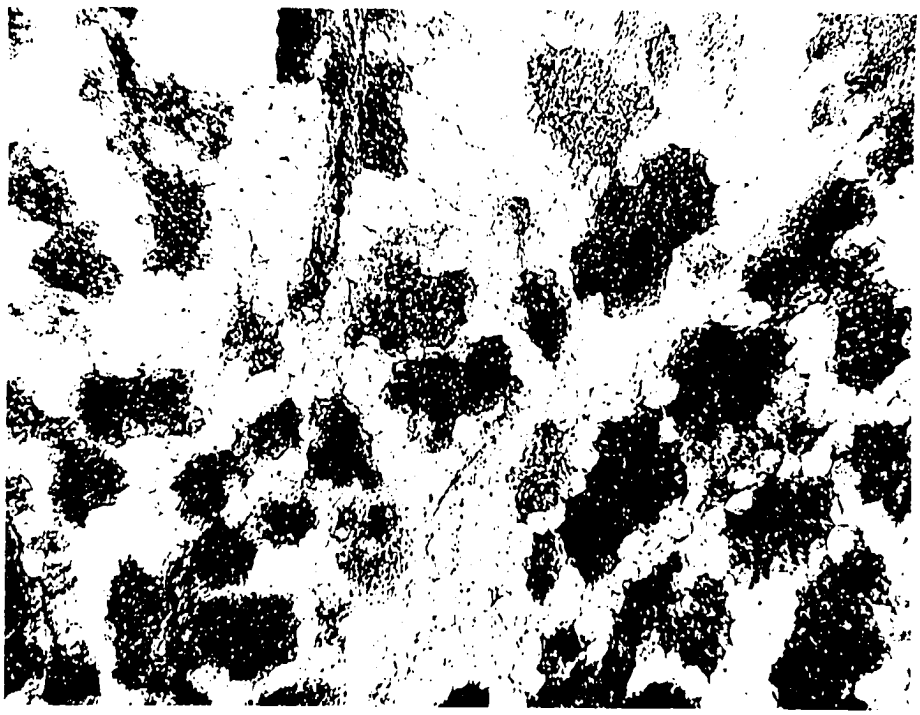

Figure 4. Mammary gland from a normal pregnant ($N_{P_{20}}$) rat

Figure 5. Mammary gland from a normal lactating (N_{L_1}) rat

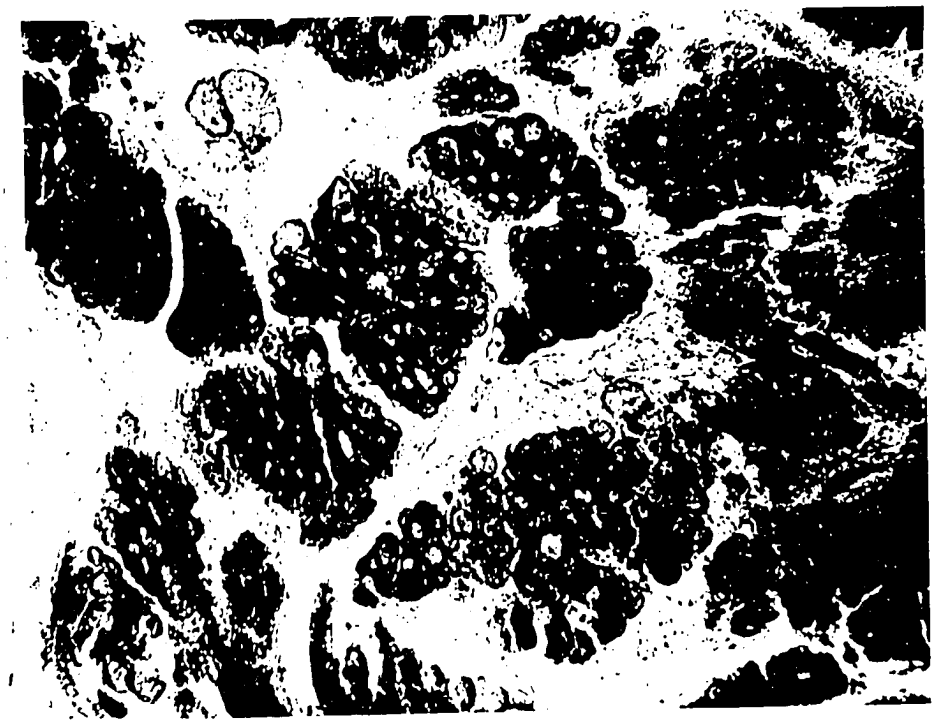

Figure 6. Mammary gland from a normal lactating (N_{L_5}) rat

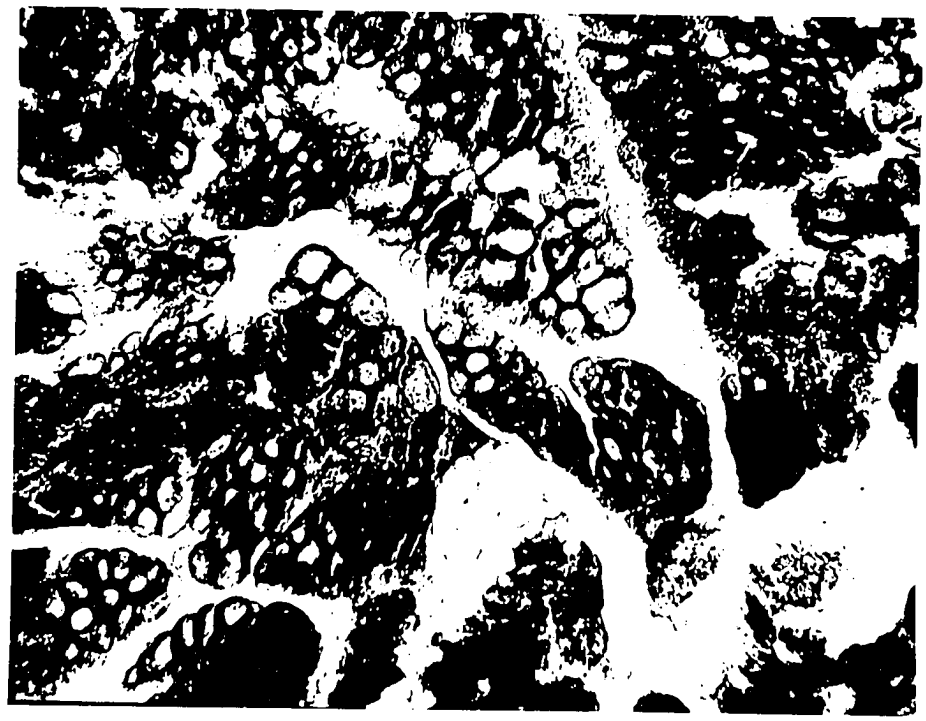

Figure 7. Mammary gland from a $N_{L_{10}}$ rat

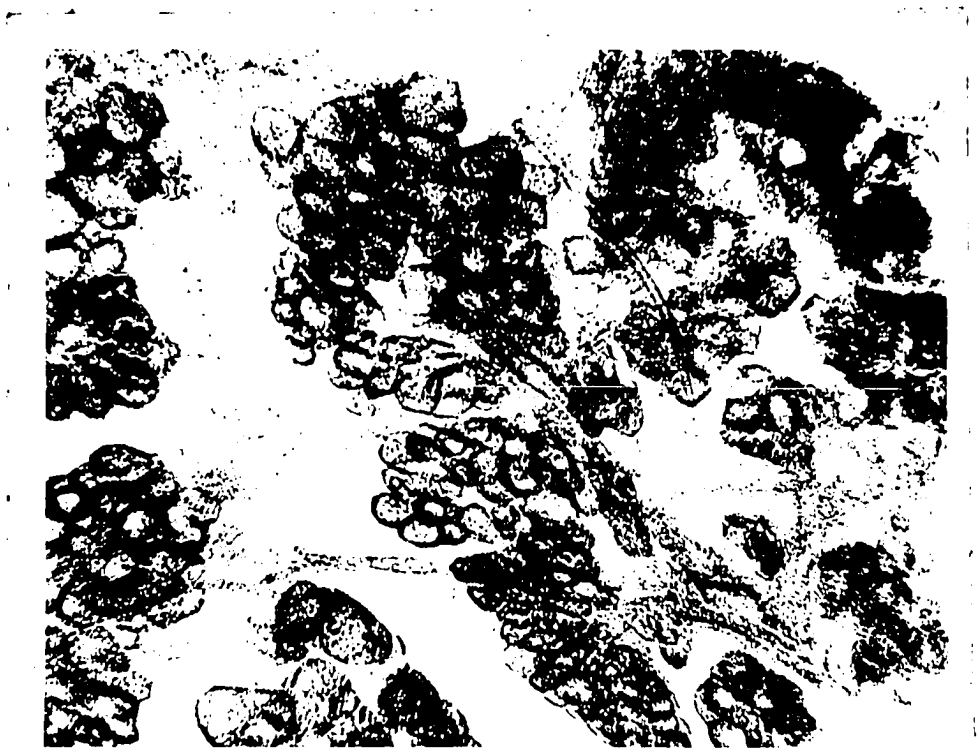

Figure 8. Mammary gland from a $N_{L_{15}}$ rat

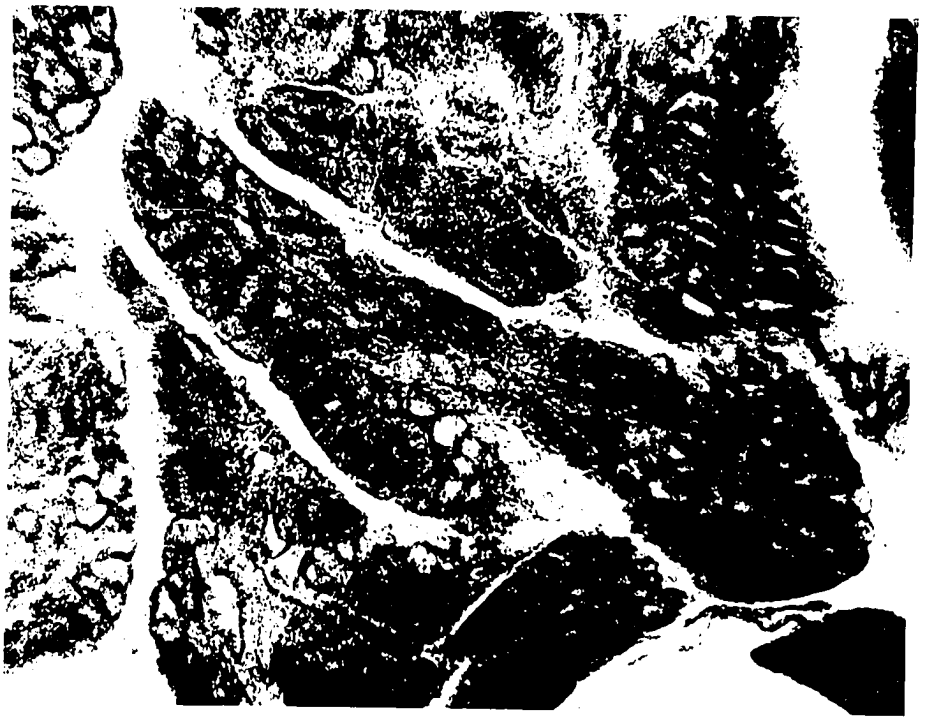

Figure 9. Mammary gland from a $N_{L_{20}}$ rat

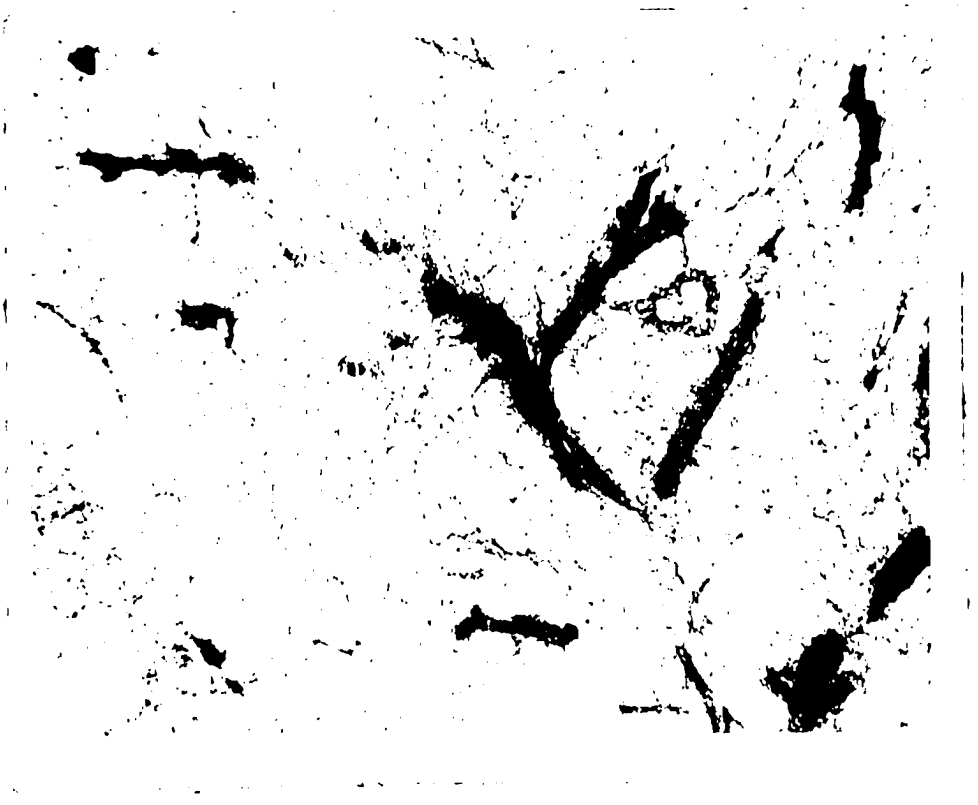

Figure 10. Mammary gland from a normal virgin (N_{P_0}) rat

presenting the results, it is necessary to explain symbolism used henceforth. All operative-treatment combinations shall be denoted in accordance with the following symbolism for simplicity:

Operative groups:

O_1 = normal control (N_C) animal,

O_2 = ovarectomized (O_X) animal,

O_3 = adrenalectomized-ovarectomized ($A_X - O_X$) animal,

O_4 = hypophysectomized-ovarectomized ($H_X - O_X$) animal,

O_5 = hypophysectomized (H_X) animal,

O_6 = adrenalectomized-hypophysectomized ($A_X - H_X$) animal,

O_7 = adrenalectomized, hypophysectomized and ovarectomized (T_X) animal.

Treatment groups:

T_1 = no treatment (N.T.)

T_2 = hormonally treated with E and P,

T_3 = hormonally treated with E, P and T,

T_4 = hormonally treated with E, P, DCA and HCA,

T_5 = hormonally treated with E, P, DCA, HCA and T,

T_6 = hormonally treated with E, P, STH, M and T,

T_7 = hormonally treated with E, P, STH and M,

T_8 = hormonally treated with E, P, DCA, HCA, STH, and T,

T_9 = hormonally treated with E, P, DCA, HCA, M and T,

T_{10}= hormonally treated with E, P, DCA, HCA, STH, M and T.

The above symbolic notations are derived from utilizing an incomplete block statistical arrangement. The symbolic notations used to denote

hormonal treatments are explained above in Table 1.

1. Normal animals (O_1)

This experimental group was set up to determine the effects of treatment, on the intact animal with a complete system of controlling mechanisms, feed-back and synergistic interrelations. The results for this group are given in Chart 2.

a. T_1 This subgroup of normal, intact animals receiving no treatment was mentioned above, as $^{N}P_{o}$, and will not be considered.

b. T_2 This subgroup received E and P at the concentrations indicated in Table 1. The means obtained as shown in Chart 2 were 3.87, 6.00, 317.1 301.2, 215.2, and 3.1 for RNA, DNA, TPN, TPNH, DPN, and DPNH, respectively. The pertinent ratio values for RNA/DNA, TPNH/TPN are DPN/DPNH were 0.64, 0.95, and 69.42, respectively. The extent of proliferation is shown in Figure 11.

c. T_3 This subgroup received E, P, and T. The extent of proliferation is shown in Figure 12. The means of the six involved substances and the ratio values were 8.71, 7.88, 1.10, 328.1, 275.6, 0.84, 290.2, 3.4, and 85.35, for RNA, DNA, RNA/DNA, TPN, TPNH, TPNH/TPN, DPN, DPNH, and DPNH/DPN, respectively. To avoid continuous reiteration of the order in which the substances and their ratios are listed, the above ordering shall be followed throughout this section, i.e., as shown in each data mean summarization table.

d. T_4 The means of the measured substances and their ratio values, as shown in Chart 2, were 6.11, 5.75, 1.06, 209.5 282.8, 1.35, 91.0, and 0.0. The ratio value of DPN/DPNH was indeterminated, since

Chart 2. Means and standard errors of means for operative group number 1 (O_1)

Treatment group	No. of experimental animals	(1) Pertinent statistic	Final animal weight (gms.)	(2) T_{RNA} per 100 gms. B.W. (mgm)	(2) T_{DNA} per 100 gms. B.W.	Ratio T_{RNA}/T_{DNA}	(3) TPN per 100 mgms. M.T.	(3) TPNH per 100 mgms. M.T.	Ratio TPNH/TPN	(3) DPN per 100 mgms. M.T.	(3) DPNH per 100 mgms. M.T.	Ratio DPN/DPNH
T_1	12	$\bar{x}$	226.9	1.12	3.20	0.35	525.8	373.3	0.71	0.0	24.6	0.0
		$s_{\bar{x}}$		±0.21	±0.29		±16.09	±14.18		0.0	±4.26	
T_2	8	$\bar{x}$	226.0	3.87	6.00	0.64	317.1	301.2	0.95	215.2	3.1	69.42
		$s_{\bar{x}}$		0.36	0.38		13.47	11.29		9.50	0.14	
T_3	8	$\bar{x}$	234.7	8.71	7.88	1.10	328.1	275.6	0.84	290.2	3.4	85.35
		$s_{\bar{x}}$		0.41	0.24		24.98	17.78		12.79	0.06	
T_4	8	$\bar{x}$	217.6	6.11	5.75	1.06	209.5	282.8	1.35	91.0	0.0	∞
		$s_{\bar{x}}$		0.35	0.43		8.72	12.77		5.48	0.0	
T_5	8	$\bar{x}$	220.5	6.87	5.97	1.15	399.0	367.1	0.92	145.8	0.0	∞
		$s_{\bar{x}}$		0.37	0.35		14.70	20.33		9.99	0.0	
T_6	8	$\bar{x}$	260.8	9.30	6.66	1.40	215.6	247.9	1.15	307.5	3.7	83.11
		$s_{\bar{x}}$		0.37	0.27		19.56	13.00		12.16	0.09	

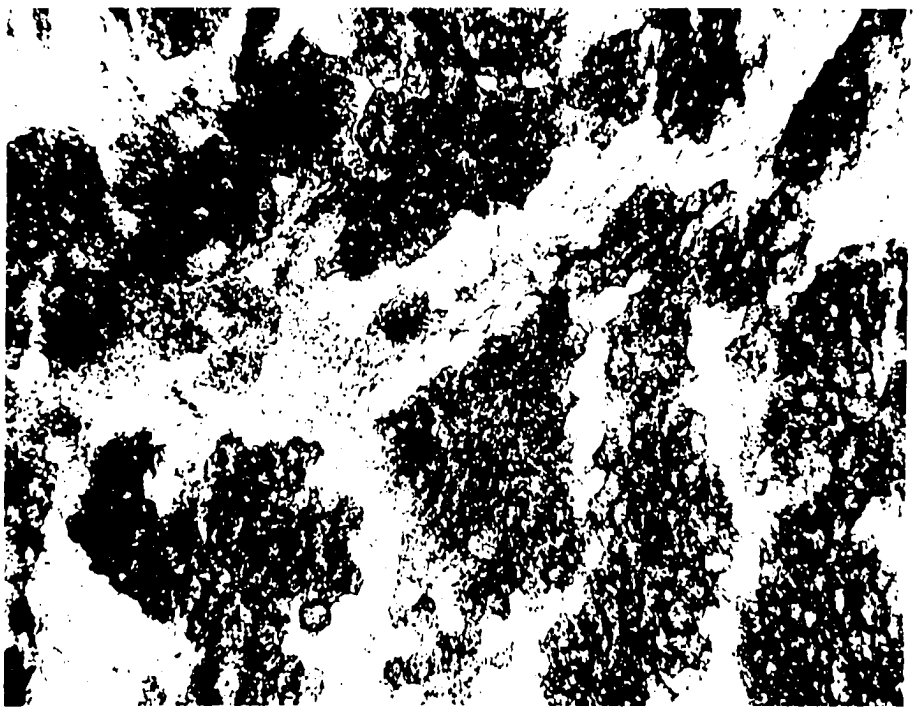

Figure 11. Mammary gland from a normal virgin (N_C) rat hormonally treated with esteone (E) and progesterone (P)

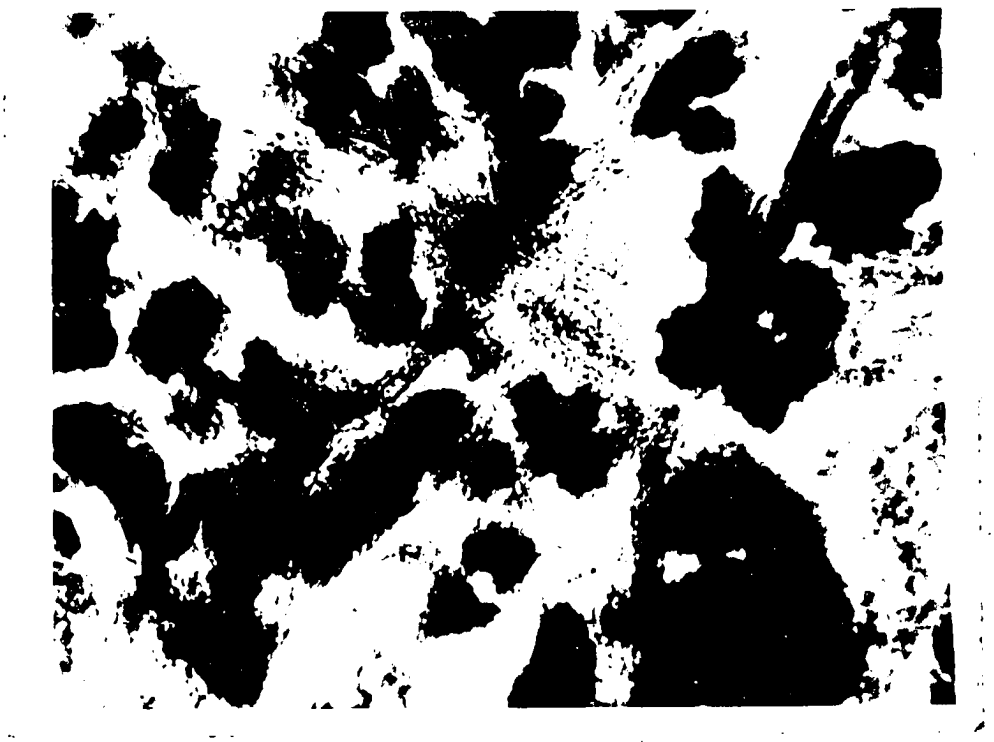

Figure 12. Mammary gland from a N_C rat hormonally treated with E, P and thyroxine (T)

the concentration of DPNH was zero; therefore it was called infinite (∞). This subtroup received as a hormonal treatment E, P, DCA, and HCA and is shown in Figure 13.

e. T_5 This subgroup received E, P, DCA, HCA, and T, see Figure 14. The respective values of the substances were 6.87, 5.97, 1.15, 399.0, 367.1, 0.92, 145.8, 0.0, and ∞.

F. T_6 This subgroup received E, P, STH, M, and T. The mean and ratio values were 9.30, 6.66, 1.40, 215.6, 247.9, 1.15, 307.5, 3.7, and 83.11. Histological results are shown in Figure 15.

2. Ovarectomized animals (O_2)

The results for this operative group are given in Chart 3.

a. T_1 These ovarectomized animals, receiving no hormonal treatment had the following mean and ratio values: 1.20, 3.12, 0.38, 432.0, 155.5, 0.36, 70.7, 8.4, and 8.42. See Figure 16.

b. T_2 This subgroup, shown histologically in Figure 17, received the hormonal injections E and P. The mean and ratio values for the measured substances were 4.78, 7.24, 0.66, 200.5, 192.5, 0.96, 15.6, 1.5, and 10.40.

c. T_3 This subgroup, as shown in Figure 18, had measured mean and ratio values of 15.40, 9.40, 0.64, 231.8, 259.6, 1.12, 19.8, 9.4, and 2.11.

d. T_4 The mean and ratio values of this subgroup were 10.31, 6.77, 1.52, 248.5, 226.1, 0.91, 0.0, 14.4, and 0.0. A histological section is shown in Figure 19.

Chart 3. Means and standard errors of means for operative group number 1 (0_2)

Treatment group	No. of experimental animals	(1) Pertinent statistic	Final animal weight (gms.)	(2) T_{RNA} per 100 gms. B.W. (mgm)	(2) T_{DNA} per 100 gms. B.W.	Ratio T_{RNA}/T_{DNA}	(3) TPN per 100 mgms. M.T.	(3) TPNH per 100 mgms. M.T.	Ratio TPNH/TPN	(3) DPN per 100 mgms. M.T.	(3) DPNH per 100 mgms. M.T.	Ratio DPN/DPNH
T_1	6	$\bar{x}$	256.6	1.20	3.12	0.38	432.0	155.5	0.36	70.7	8.4	8.42
		$s_{\bar{x}}$		±0.33	±0.13		±19.23	±21.32		±3.69	±1.15	
T_2	19	$\bar{x}$	250.5	4.78	7.24	0.66	200.5	192.5	0.96	15.6	1.5	10.40
		$s_{\bar{x}}$		0.37	0.46		17.49	16.39		2.14	0.02	
T_3	17	$\bar{x}$	225.1	15.40	9.40	0.64	231.8	259.6	1.12	19.8	9.4	2.11
		$s_{\bar{x}}$		0.29	0.27		11.78	15.59		2.71	0.64	
T_4	19	$\bar{x}$	228.9	10.31	6.77	1.52	248.5	226.1	0.91	0.0	14.4	0.0
		$s_{\bar{x}}$		0.23	0.38		14.07	24.98		0.0	1.01	
T_7	8	$\bar{x}$	236.5	15.65	8.42	1.86	236.7	416.6	1.76	15.9	7.8	2.04
		$s_{\bar{x}}$		0.46	0.29		12.45	17.12		1.17	0.76	

Figure 13. Mammary gland from a N_6 rat hormonally treated with E, P, desoxycorticosterone (DCA) and hydrocorticosterone (HCA)

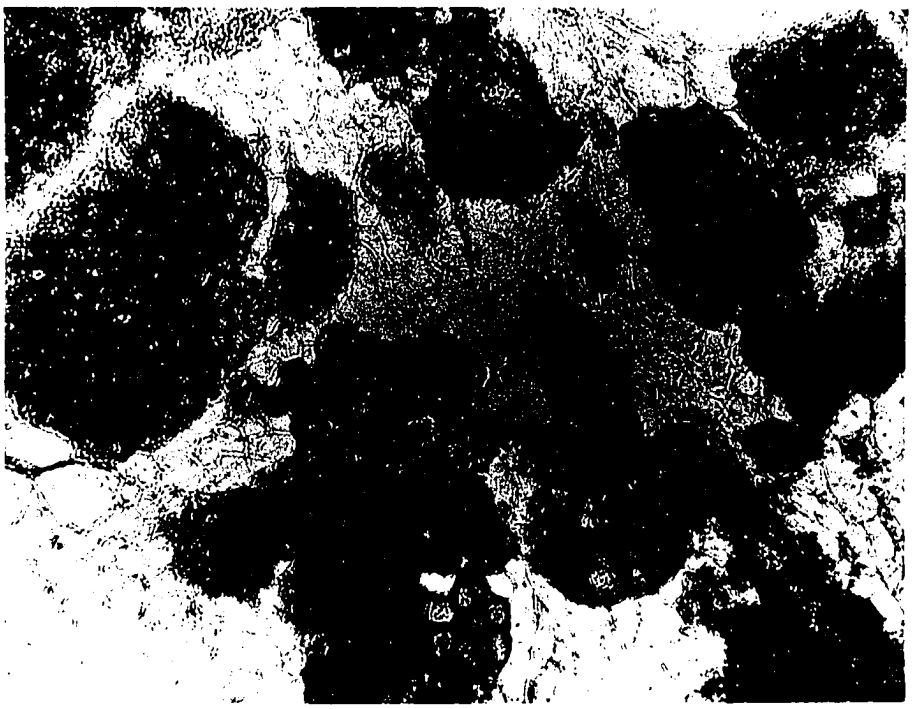

Figure 14. Mammary gland from a N_C rat hormonally treated with E, P, DCA, HCA and T

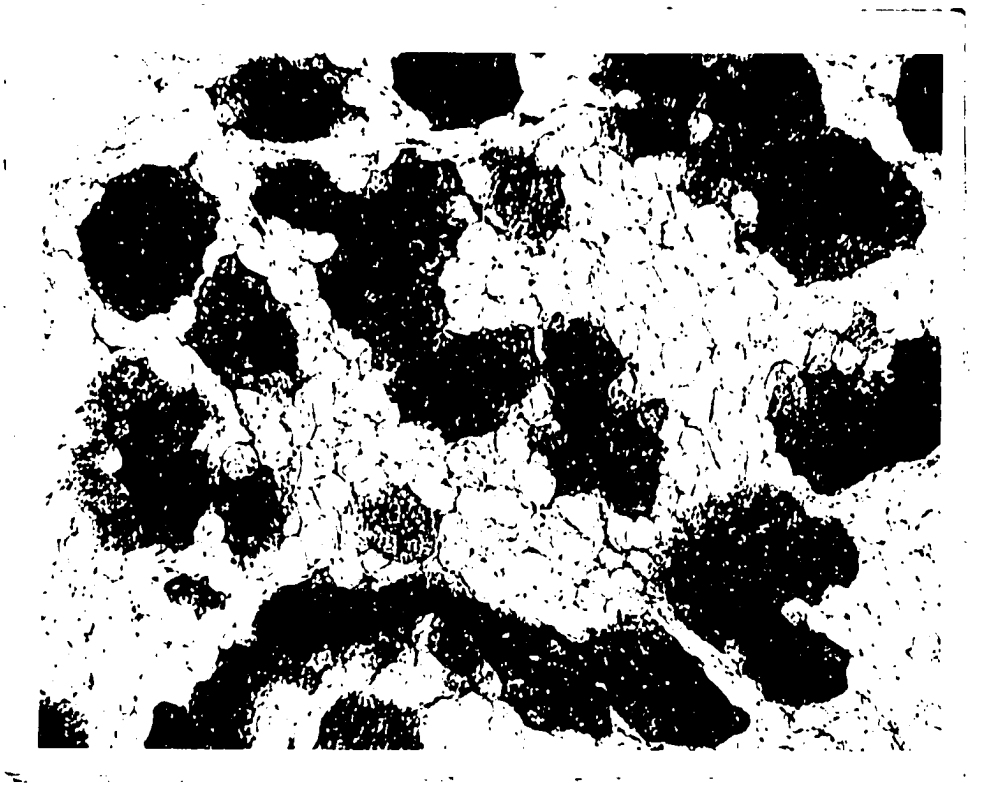

Figure 15. Mammary gland from a N_C rat hormonally treated with E, P, growth hormone (STH), prolactin (M) and T

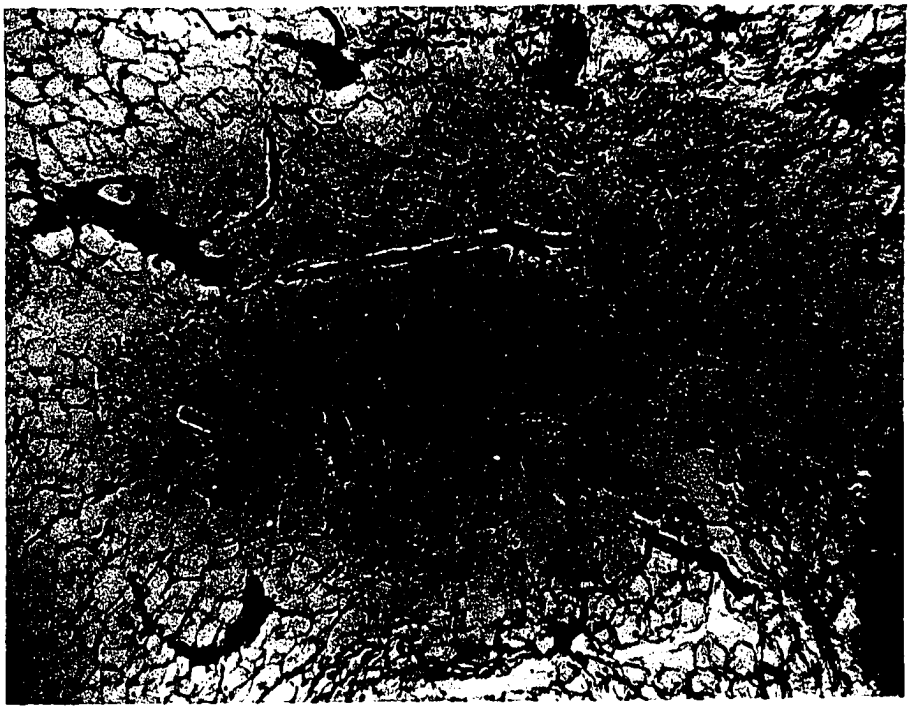

Figure 16. Mammary gland from an ovarectomized (0_X) rat receiving no hormonal treatment

Figure 17. Mammary gland from an 0_X rat hormonally treated with E and P

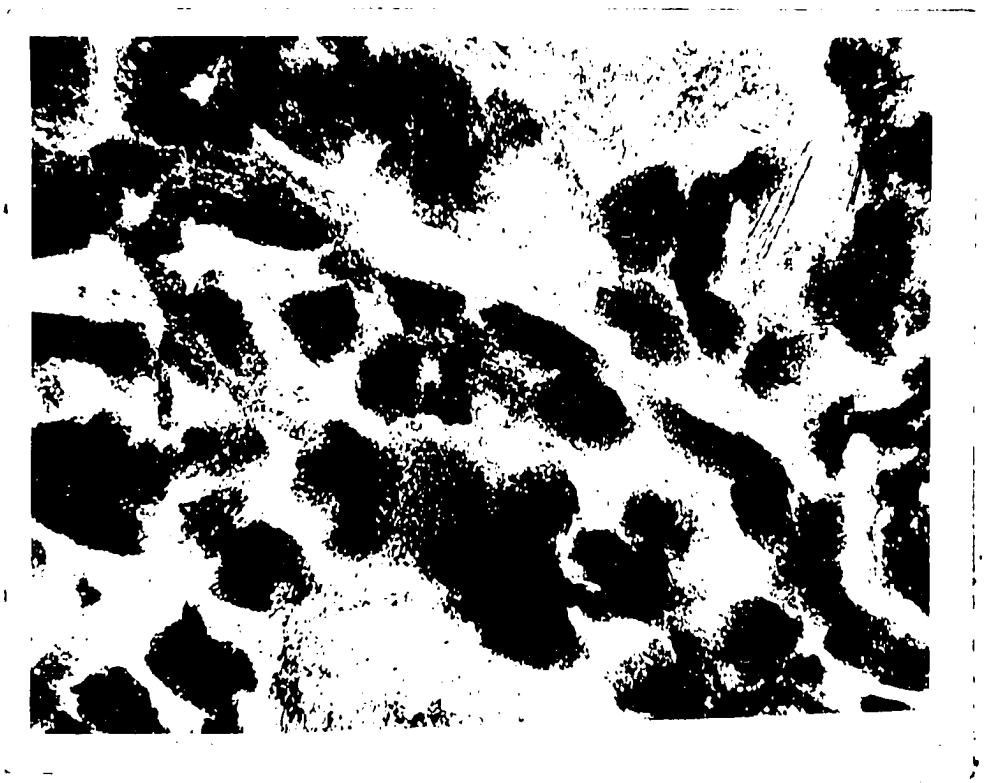

Figure 18. Mammary gland from an 0_X rat hormonally treated with E, P and T

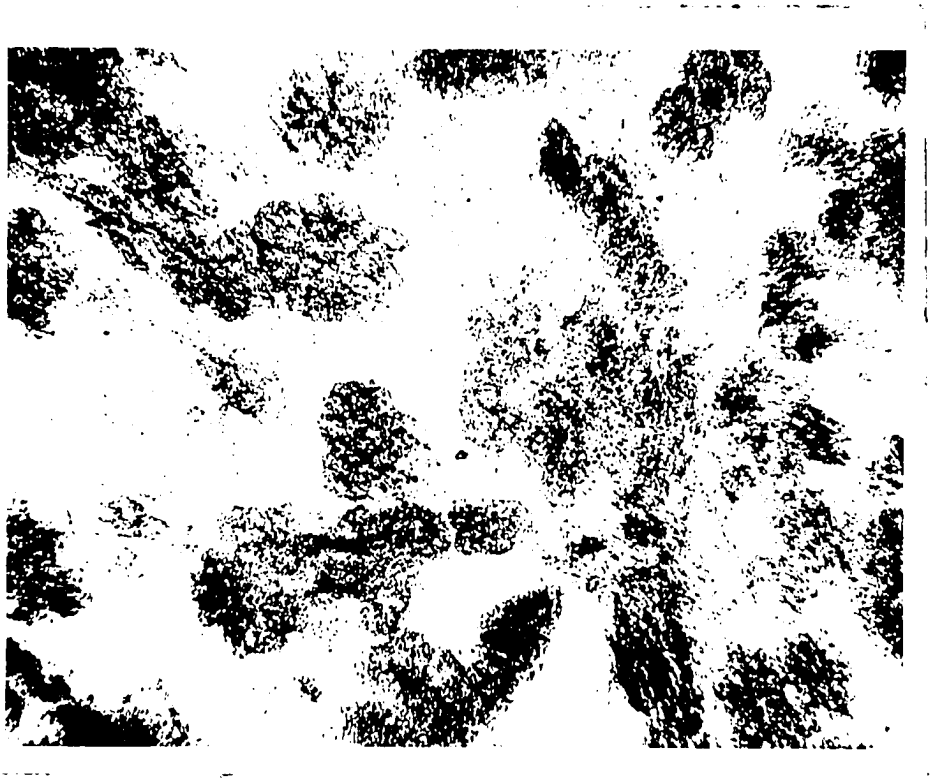

Figure 19. Mammary gland from an 0_X rat hormonally treated with E, P, D and H

e. T_7 This subgroup, receiving E, P, STH, and M, have mean and ratio values of 15.65, 8.42, 1.86, 236.7, 416.6, 1.76, 15.9, 7.8, and 2.04. See Figure 20.

3. Adrenalectomized-ovarectomized animals (O_3)

a. T_1 This subgroup had mean and ratio values, as given in Chart 4, of 4.06, 3.95, 1.02, 176.8, 47.7, 0.27, 6.5, 0.0 and See Figure 21 for a histological section.

b. T_2 The mean and ratio values of this subgroup were 4.61, 7.33, 0.63, 192.8, 79.0, 0.41, 14.9, 6.7, and 2.22 for RNA, DNA, RNA/DNA, TPN, TPNH, TPNH/TPN, DPN, DPNH, and DPN/DPNH, respectively. The extent of proliferation is shown in Figure 22.

c. T_4 This subgroup received E, P, DCA, and HCA, had mean and ratio values of 11.65, 7.17, 1.62, 310.7, 183.3, 0.59, 0.0, 1.33, and 0.0. (See Figure 23).

d. T_5 This subgroup, as shown in Figure 24, had mean and ratio values of 14.62, 8.75, 1.63, 254.5, 193.4, 0.76, 21.6, 49.6, and 0.44 To reiterate, this subgroup received as hormonal therapy E, P, DCA, HCA, and T.

4. Hypophysectomized-ovarectomized animals (O_4)

The results for the operative group are to be found in Chart 5.

a. T_1 This experimental operative-treatment subgroup, receiving no hormonal treatment, had mean and ratio values of 1.65, 1.97, 0.84, 761.7, 236.7, 0.31, 15.7, 0.0 and ∞. (See Figure 25).

Chart 4. Means and standard errors of means for operative group number 1 (0_3)

Treatment group	No. of experimental animals	(1) Pertinent statistic	Final animal weight (gms.)	(2) T_{RNA} per 100 gms. B.W. (mgm)	(2) T_{DNA} per 100 gms. B.W.	Ratio T_{RNA}/T_{DNA}	(3) TPN per 100 mgms. M.T.	(3) TPNH per 100 mgms. M.T.	Ratio TPNH/TPN	(3) DPN per 100 mgms. M.T.	(3) DPNH per 100 mgms. M.T.	Ratio DPN/DPNH
T_1	6	$\bar{x}$	206.5	4.06	3.95	1.02	176.8	47.7	0.27	6.5	0.0	∞
		$s_{\bar{x}}$		0.31	0.27		14.24	2.27		0.41	0.0	
T_2	8	$\bar{x}$	225.6	4.61	7.33	0.63	192.8	79.0	0.41	14.9	6.7	2.22
		$s_{\bar{x}}$		0.22	0.46		16.43	5.16		0.67	1.01	
T_4	15	$\bar{x}$	229.2	11.65	7.17	1.62	310.7	183.3	0.59	0.0	11.3	0.0
		$s_{\bar{x}}$		0.33	0.21		19.13	9.51		0.0	0.73	
T_5	8	$\bar{x}$	231.5	14.62	8.75	1.63	254.5	193.4	0.76	21.6	49.6	0.44
		$s_{\bar{x}}$		0.36	0.51		11.52	8.72		1.79	2.15	

Chart 5. Means and standard errors of means for operative group number 1 (0_4)

Treatment group	No. of experimental animals	(1) Pertinent statistic	Final animal weight (gms.)	(2) T_{RNA} per 100 gms. B.W. (mgm)	(2) T_{DNA} per 100 gms. B.W.	Ratio T_{RNA}/T_{DNA}	(3) TPN per 100 mgms. M.T.	(3) TPNH per 100 mgms. M.T.	Ratio TPNH/TPN	(3) DPN per 100 mgms. M.T.	(3) DPNH per 100 mgms. M.T.	Ratio DPN/DPNH
T_1	6	$\bar{x}$	215.7	1.65	1.97	0.84	761.7	236.7	0.31	15.7	0.0	∞
		$s_{\bar{x}}$		±0.25	±0.36		±27.61	±13.65		±3.51	0.0	
T_2	8	$\bar{x}$	227.4	1.36	2.05	0.66	465.6	451.6	0.97	0.0	27.6	0.0
		$s_{\bar{x}}$		0.51	0.48		13.06	27.92		0.0	3.27	
T_3	8	$\bar{x}$	229.7	0.87	1.87	0.47	502.5	356.8	0.71	29.6	12.7	2.33
		$s_{\bar{x}}$		0.33	0.27		13.72	21.16		3.16	2.64	
T_6	8	$\bar{x}$	236.5	2.79	2.85	0.97	229.7	296.3	1.29	29.5	6.7	4.40
		$s_{\bar{x}}$		0.31	0.31		9.27	20.57		7.09	0.92	

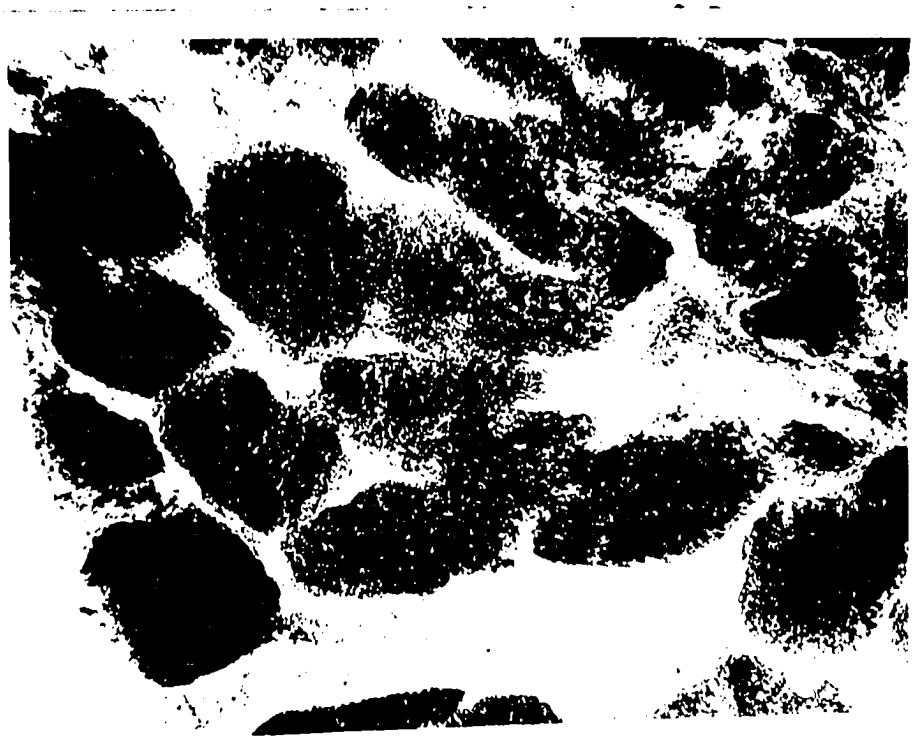

Figure 20. Mammary gland from an O_X rat hormonally treated with E, P, STH and M

Figure 21. Mammary gland from an adrenalectomized (A_X)-O_X rat receiving no hormonal treatment

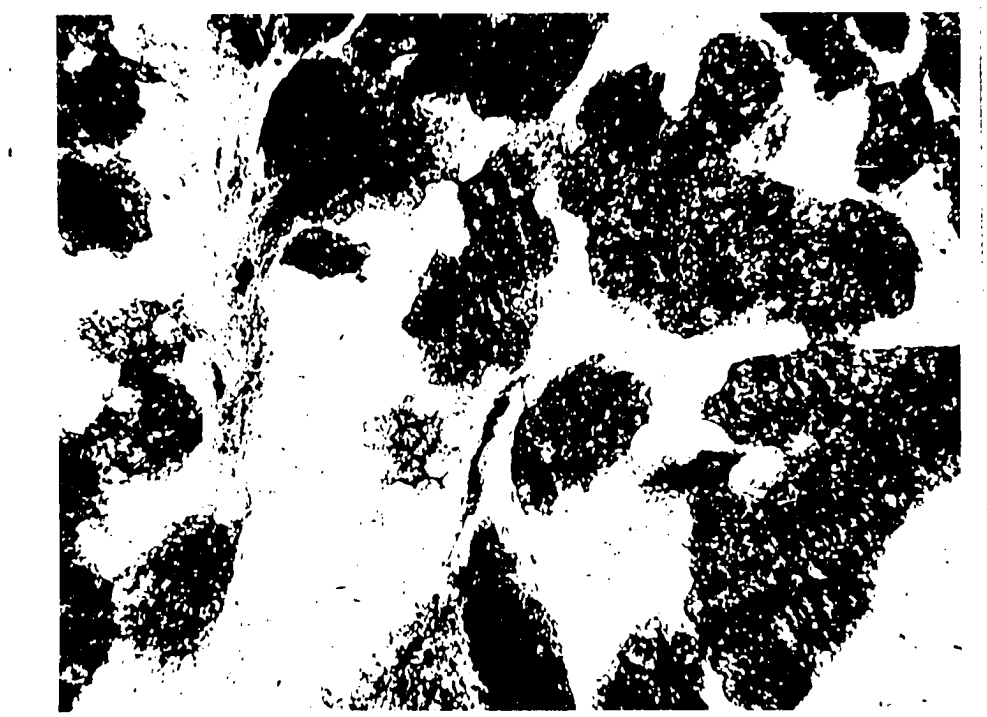

Figure 22. Mammary gland from an A_X-0_X rat hormonally treated with E and P

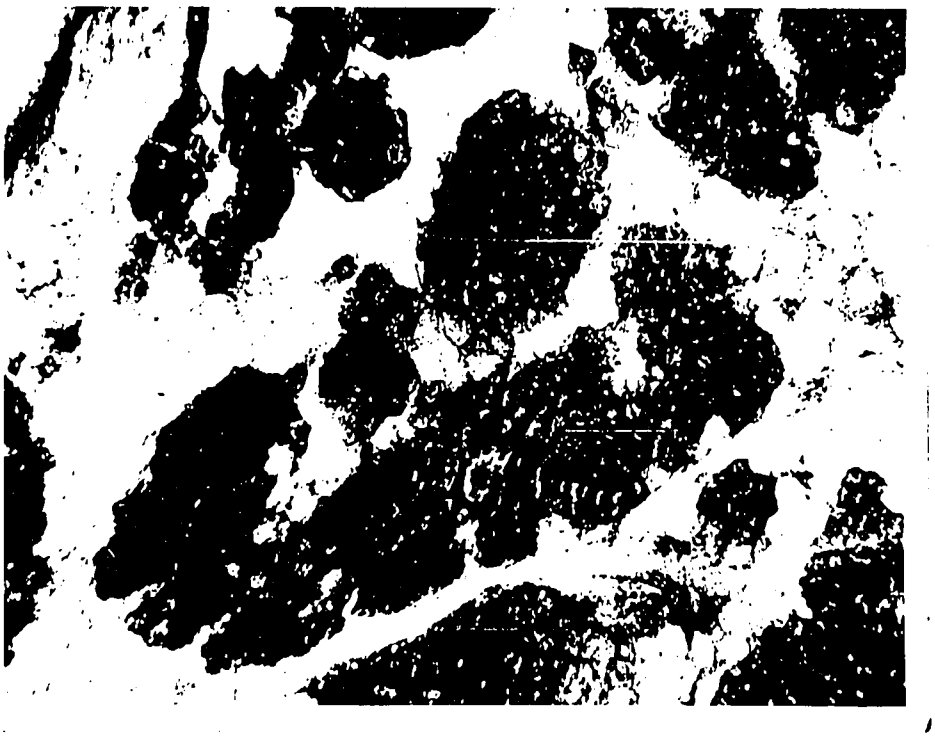

Figure 23. Mammary gland from an A_X-0_X rat hormonally treated with E, P, D and H

Figure 24. Mammary gland from an A_X-O_X rat hormonally treated with E, P, D, H and T

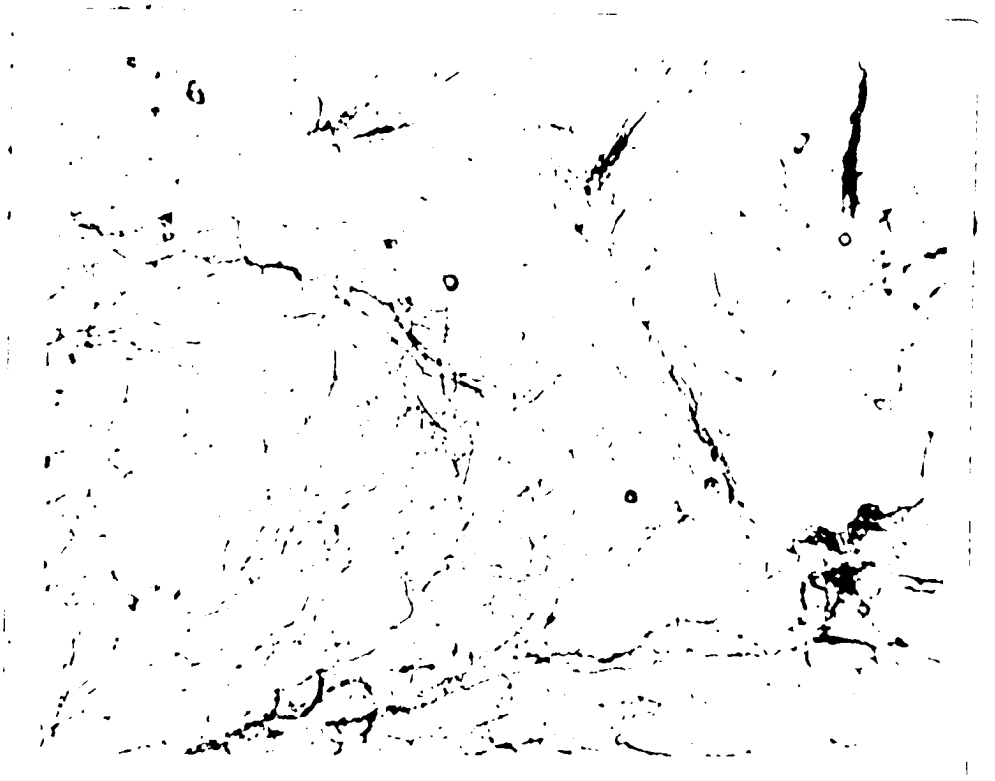

Figure 25. Mammary gland from a hypophysectomized (H_X)-O_X rat receiving no hormonal treatment

Figure 26. Mammary gland from a typical H_X-O_X rat for all hormonal treatments

b. T_2 This subgroup, receiving as hormonal therapy E and P, had mean and ratio values of 1.36, 2.05, 0.66, 464.6, 541.6, 0.97, 0.0, 27.6, and 0.0. Due to similarities in the extent of glandular proliferation, the remainder of this group is best represented histologically by Figure 26.

c. T_3 This subgroup had mean and ratio values of 0.87, 1.87, 0.47, 502.5, 356.8, 0.71, 29.6, 12.7, and 2.33. The hormonal treatment consisted of E, P, and T.

d. T_6 The mean and ratio values of this subgroup, receiving E, P, STH, M, and T, were 2.79, 2.85, 0.97, 229.7, 1.29, 29.5, 6.7, 4.40

5. Hyposectomized animals (O_5)

The results of this operative group are given in Chart 6.

a. T_1 This subgroup as shown in Figure 27, had mean and ratio values of 1.39, 1.66, 0.84, 637.7, 223.2, 0.35, 19.5, 5.1 and 3.82.

b. T_2 The mean and ratio values of this subgroup were 1.36, 1.72, 0.79, 816.3, 375.5, 0.48, 36.1, 0.2, and 3.92. Again, due to histological similarities, the remainder of this group is best illustrated by Figure 28.

c. T_3 This subgroup had mean and ratio values of 1.58, 1.65, 0.96, 705.6, 190.5, 027, 29.5, 7.6, and 3.88.

d. T_5 This subgroup had mean and ratio values of 0.77, 1.45, 0.53, 427.6, 188.1, 0.44, 31.7, 12.9, and 2.46. The hormonal treatment of this subgroup was E, P, DCA, HCA, and T.

e. T_6 The mean and ratio values of this subgroup were 2.39,

Chart 6. Means and standard errors of means for operative group number 1 (0_5)

Treatment group	No. of experimental animals	(1) Pertinent statistic	Final animal weight (gms.)	(2) T_{RNA} per 100 gms. B.W. (mgm)	(2) T_{DNA} per 100 gms. B.W.	Ratio T_{RNA}/T_{DNA}	(3) TPN per 100 mgms. M.T.	(3) TPNH per 100 mgms. M.T.	Ratio TPNH/TPN	(3) DPN per 100 mgms. M.T.	(3) DPNH per 100 mgms. M.T.	Ratio DPN/DPNH
T_1	6	$\bar{x}$	207.5	1.39	1.66	0.84	637.7	223.2	0.35	19.5	5.1	3.82
		$s_{\bar{x}}$		±0.37	±0.15		±21.65	±15.09		±3.62	±0.07	
T_2	8	$\bar{x}$	200.2	1.36	1.72	0.79	816.3	375.5	0.47	36.1	9.2	3.92
		$s_{\bar{x}}$		0.41	0.36		42.15	27.28		4.01	0.41	
T_3	8	$\bar{x}$	197.0	1.58	1.65	0.96	705.6	190.5	0.27	29.5	7.6	3.88
		$s_{\bar{x}}$		0.29	0.42		31.27	13.17		3.97	0.39	
T_5	8	$\bar{x}$	157.2	0.77	1.45	0.53	427.6	188.1	0.44	31.7	12.9	2.46
		$s_{\bar{x}}$		0.46	0.21		19.88	15.42		4.15	0.46	
T_6	9	$\bar{x}$	232.4	2.39	2.42	0.99	316.5	364.0	1.15	31.6	5.4	5.85
		$s_{\bar{x}}$		0.41	0.35		17.52	27.49		2.46	0.15	

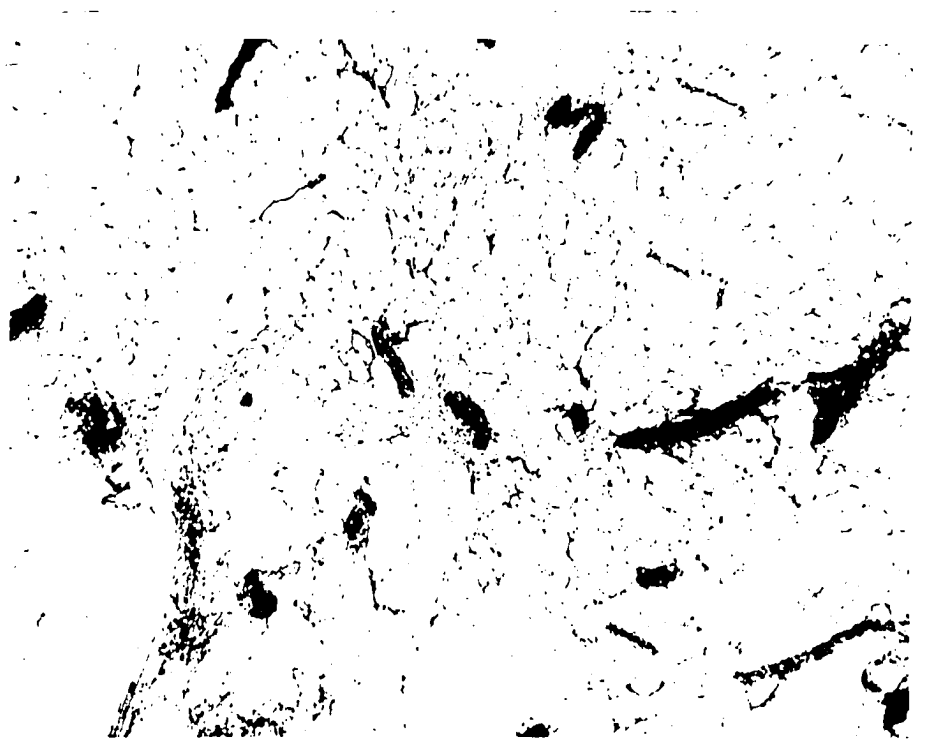

Figure 27. Mammary gland from a H_X rat receiving no hormonal treatment

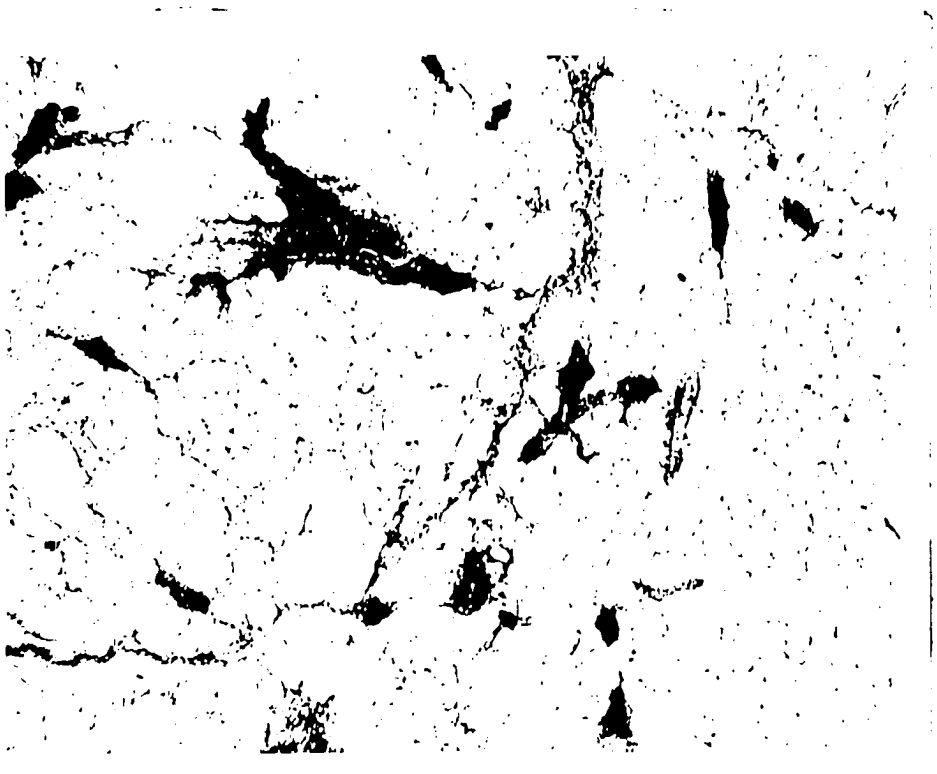

Figure 28. Mammary gland from a typical H_X rat for all hormonal treatments

2.42, 0.99, 316.5, 364.0, 1.15, 31.6, 5.4, and 5.85.

6. Adrenalectomized-hypophysectomized animals (O_6)

The results of this operative group of animals are given in Chart 7. This group consisted of only one treatment subgroup, T_1, the mean and ratio values of this subgroup were 1.29, 1.51, 0.85, 629.5, 163.7, 0.26, 15.1, 1.7, and 8.88. (See Figure 29).

7. Adrenalectomized-hypophysectomized-ovarectomized animals (O_7)

The results for this operative group are shown in Chart 8.

a. T_1 The mean and ratio values of this subgroup were 1.45, 2.05, 0.71, 765.8, 275.7, 0.36, 0.0, 39.7, and 0.0. For histological representation, see Figure 30.

b. T_2 The mean and ratio values of this subgroup were 1.11, 1.90, 0.58, 775.6, 219.6, 0.28, 0.0, 4.4, and 0.0. Treatments T_2 through T_5 are typical of the extent of proliferation as shown in Figure 21.

c. T_4 This subgroup had mean and ratio values of 1.05, 1.55, 0.68, 975.9, 351.3, 0.36, 28.9, 7.0, and 4.13.

d. T_5 The mean and ratio values of this subgroup were 0.63, 1.67, 0.38, 756.0, 310.0, 0.41, 39.9, 11.4, and 3.50.

e. T_8 This subgroup, receiving as hormonal therapy E, P, DCA, HCA, M, and T, had mean and ratio values of 3.69, 3.54, 1.04, 559.7, 442.2, 0.79, 25.4, 3.2, and 7.94. Proliferation is shown histologically in Figure 32.

f. T_9 This subgroup had mean and ratio values of 6.01, 6.03,

Chart 7. Means and standard errors of means for operative group number 1 (0_6)

Treatment group	No. of experimental animals	(1) Pertinent statistic	Final animal weight (gms.)	(2) T_{RNA} per 100 gms. B.W. (mgm)	(2) T_{DNA} per 100 gms. B.W.	Ratio T_{RNA}/T_{DNA}	(3) TPN per 100 mgms. M.T.	(3) TPNH per 100 mgms. M.T.	Ratio TPNH/TPN	(3) DPN per 100 mgms. M.T.	(3) DPNH per 100 mgms. M.T.	Ratio DPN/DPNH
T_1	6	$\bar{x}$	215.6	1.29	1.51	0.85	629.5	163.7	0.26	15.1	1.7	8.88
		$s_{\bar{x}}$		±0.15	±0.21		±26.30	±10.15		±0.25	±0.01	

Chart 8. Means and standard errors of means for operative group number 1 (0_7)

Treatment group	No. of experimental animals	(1) Pertinent statistic	Final animal weight (gms.)	(2) T_{RNA} per 100 gms. B.W. (mgm)	(2) T_{DNA} per 100 gms. B.W.	Ratio T_{RNA}/T_{DNA}	(3) TPN per 100 mgms. M.T.	(3) TPNH per 100 mgms. M. T.	Ratio TPNH/TPN	(3) DPN per 100 mgms. M.T.	(3) DPNH per 100 mgms. M.T.	Ratio DPN/DPNH
T_1	6	$\bar{x}$	227.6	1.45	2.05	0.71	765.8	275.7	0.36	0.0	39.7	0.0
		$s_{\bar{x}}$		±0.21	±0.15		±19.73	±13.51		0.0	±1.72	
T_2	8	$\bar{x}$	173.3	1.11	1.90	0.58	775.6	219.6	0.28	0.0	4.4	0.0
		$s_{\bar{x}}$		0.36	0.17		27.27	14.26		0.0	0.46	
T_4	8	$\bar{x}$	182.6	1.05	1.55	0.68	975.9	351.3	0.36	28.9	7.0	4.13
		$s_{\bar{x}}$		0.15	0.21		31.15	16.42		1.79	0.71	
T_5	8	$\bar{x}$	128.5	0.63	1.67	0.38	756.0	310.0	0.41	39.9	11.4	3.50
		$s_{\bar{x}}$		0.25	0.27		31.71	16.79		3.51	1.57	
T_8	8	$\bar{x}$	128.5	3.69	3.54	1.04	559.7	442.2	0.79	25.4	3.2	7.94
		$s_{\bar{x}}$		0.34	0.23		26.92	15.29		3.36	0.51	
T_9	8	$\bar{x}$	107.1	6.01	6.03	1.00	427.6	517.4	1.21	19.6	2.9	6.76
		$s_{\bar{x}}$		0.23	0.19		21.42	27.71		2.15	0.19	
T_{10}^{10}	10	$\bar{x}$	215.8	2.74	2.91	0.94	146.5	301.8	2.06	15.5	2.5	6.20
		$s_{\bar{x}}$		0.19	0.37		11.15	21.09		0.59	0.27	
T_{10}^{19}	8	$\bar{x}$	236.2	2.79	2.68	1.04	159.6	349.5	2.19	14.6	2.7	5.41
		$s_{\bar{x}}$		0.27	0.15		19.25	22.15		0.67	0.71	
T_{10}	18	$\bar{x}$	224.0	2.76	2.80	0.99	152.5	325.7	2.14	14.8	2.6	5.69
		$s_{\bar{x}}$		0.25	0.31		17.01	22.07		0.63	0.51	

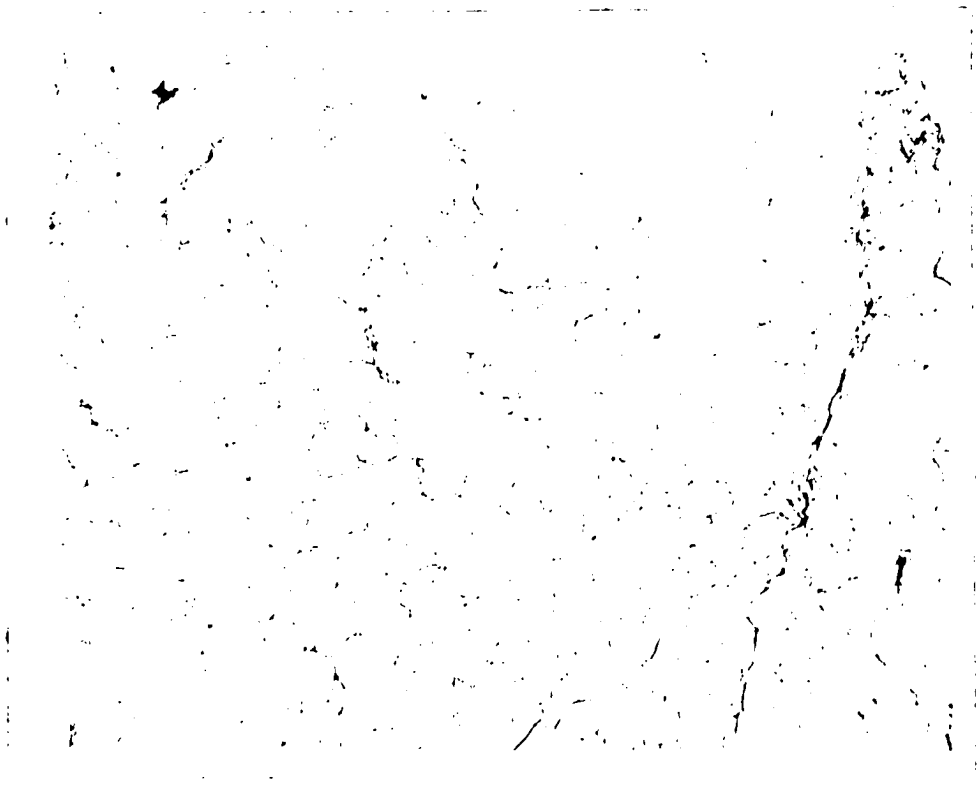

Figure 29. Mammary gland from an A_X-H_X rat receiving no hormonal treatment

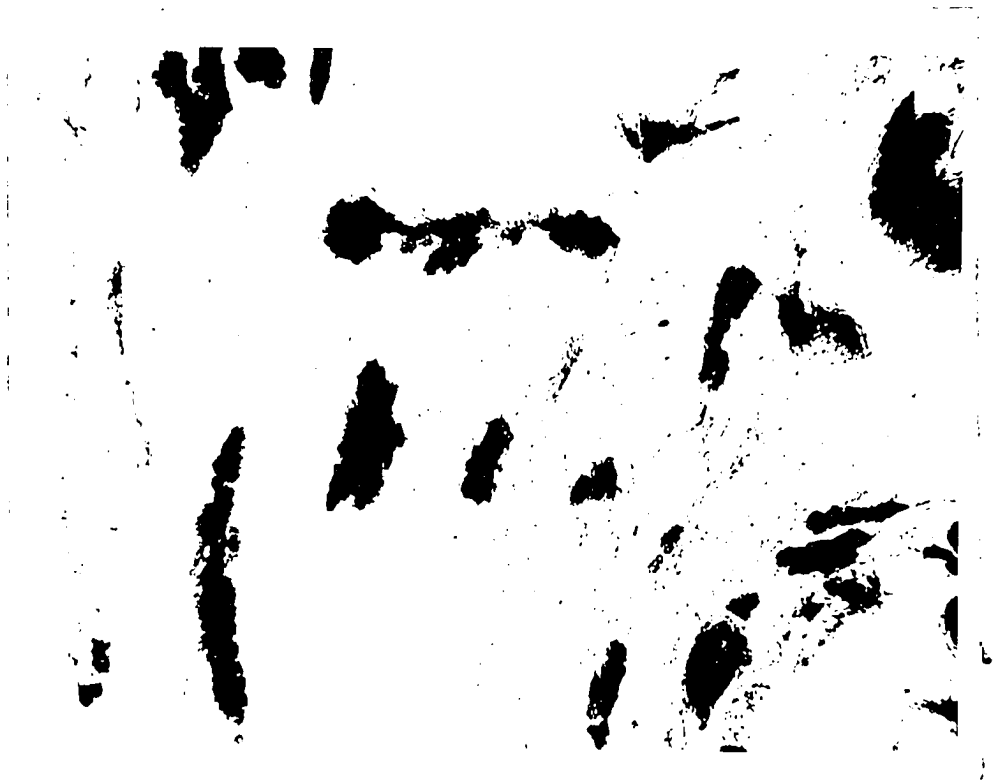

Figure 30. Mammary gland from a A_X-H_X-O_X (T_X) rat receiving no hormonal treatment

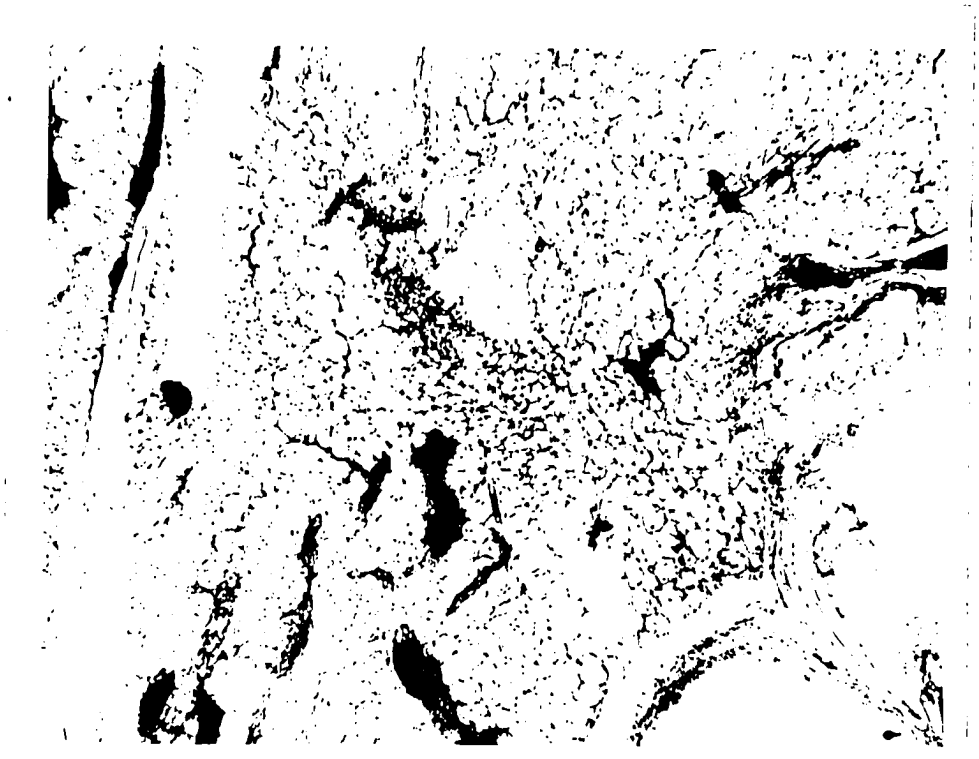

Figure 31. Mammary gland from a typical T_X rat hormonally treated with E and P, E, P, DCA and HCA or E, P, DCA, HCA and T

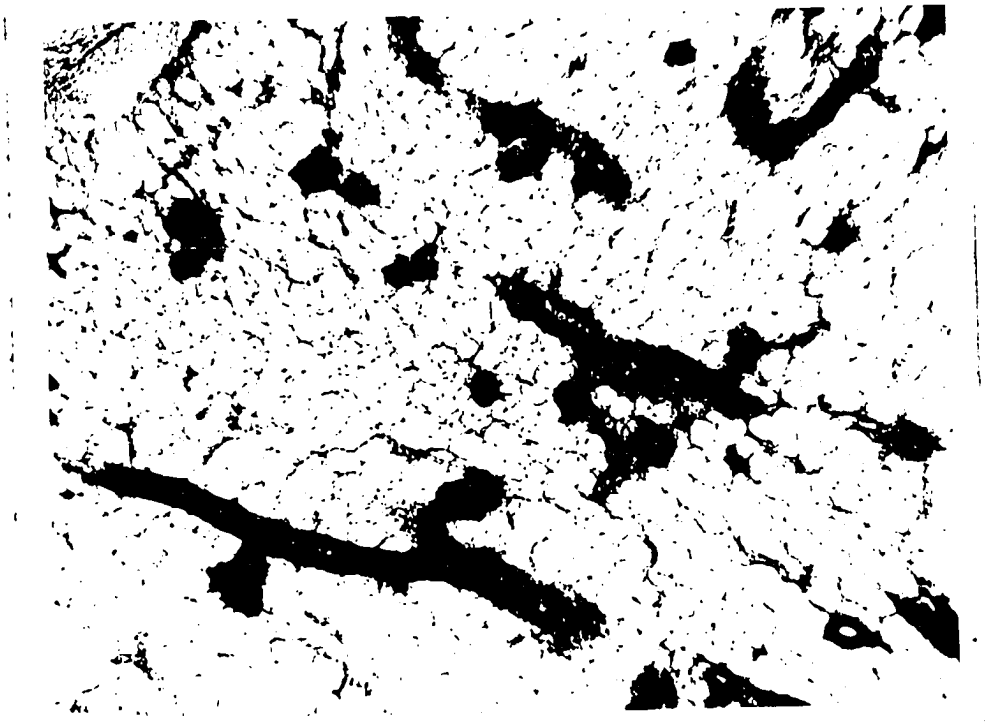

Figure 32. Mammary gland from a T_X rat hormonally treated with E, P, DCA, HCA, STH and T

1.00, 427.6, 517.4, 1.21, 19.6, 2.9, and 6.76. The hormonal therapy of this subgroup was E, P, DCA, HCA, M, and T. (See Figure 33).

g. $\underline{T_{10}^{10}}$, $\underline{T_{10}^{19}}$, and $\underline{T_{10}}$

The purpose of defining two separate subgroups, T_{10}^{10} and T_{10}^{19}, as given in Chart 8, was to test the differences due to the injection of growth hormone (STH) for 10 and 19 days. The experiment was designed to test the effect of any protein-antibody reaction with prolonged injection of STH. A comparison of the RNA values, using the "t" test (166), was found to be insignificant, probability (Prob.) ≤ 0.50. The probabilities when comparing DNA, TPN, TPNH, DPN, and DPNH for the two injection periods were Prob. ≤ 0.50, Prob. ≤ 0.50, Prob. ≤ 0.10, Prob. ≤ 0.30, and Prob. ≤ 0.50, respectively; therefore, showing no difference in any of the six measured substance for the two different injection periods. The data from these two experimental periods were pooled, since no differences existed, and are represented as one operative-treatment subgroup, $O_7 - T_{10}$, as shown in Chart 8. The mean and ratio values of this subgroup were 2.76, 2.80, 0.99, 152.5, 325.7, 2.14, 14.8, 2.6, and 5.69. The complete hormonal therapy of this combined subgroup was E, P, DCA, HCA, STH, M, and T. Visually, the two groups are shown in Figures 34 and 35.

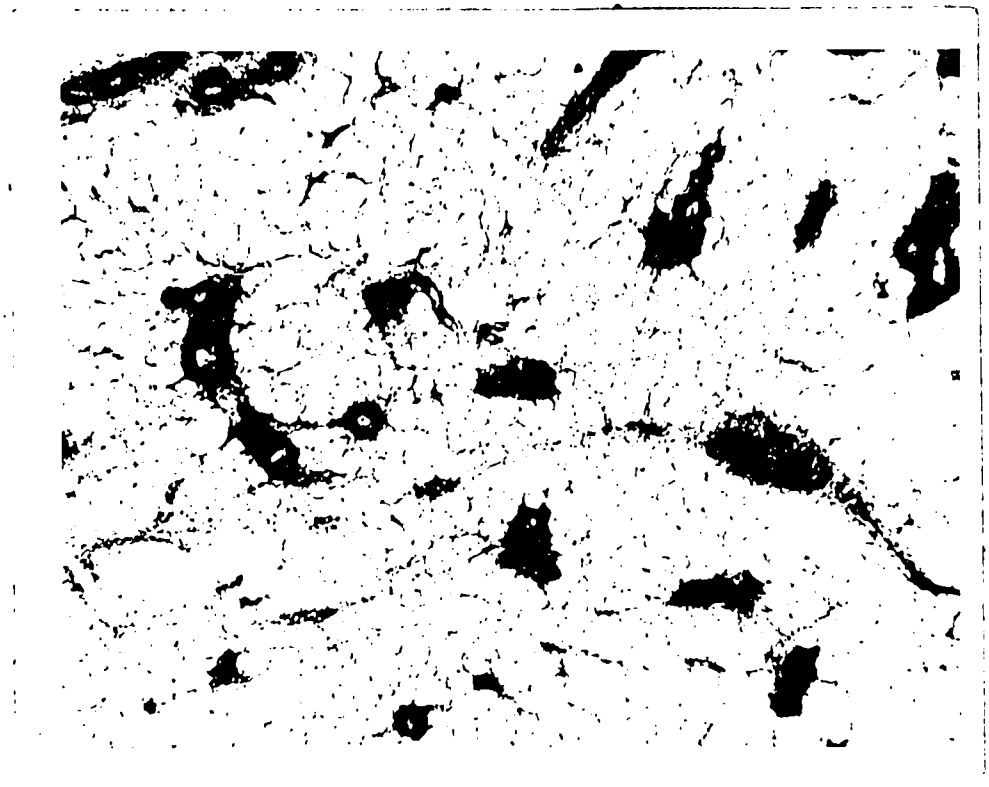

Figure 33. Mammary gland from a T_X rat hormonally treated with E, P, DCA, HCA, M and T

Figure 34. Mammary gland from a T_X rat hormonally treated for ten days with E, P, DCA, HCA, STH, M and T

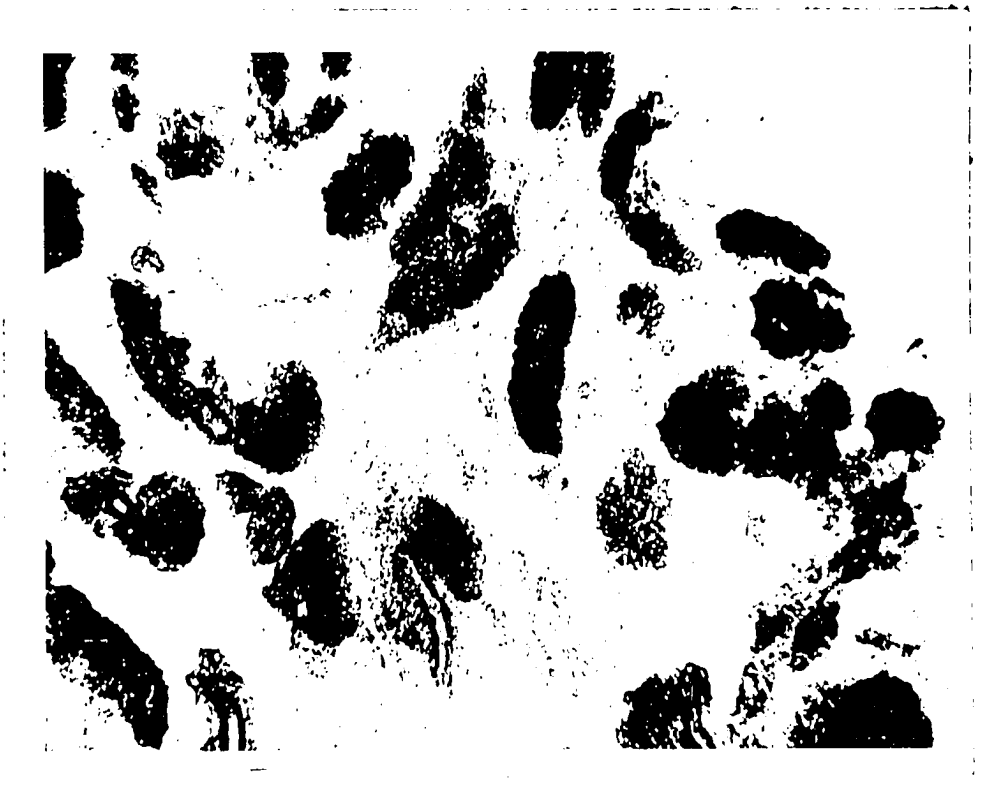

Figure 35. Mammary gland from a T_X rat hormonally treated for nineteen days with E, P, DCA, HCA, STH, M and T

V. STATISTICAL EVALUATION

A. Natural Mammogenesis

A plot of the mean values for normal pregnancy and lactation for each of the measured substances and the three ratio values through time are shown in Figures 36 through 38. These figures illustrate the sequential or time-trend variation of the normal pregnant ($^{N}P_{i}$) and the normal lactating ($^{N}L_{i}$) animal, where P_{i} or L_{i} indicates the day of pregnancy or lactation, respectively, when the values were sampled. Figure 36 shows as a scattergram the variation of total RNA and DNA per 100 grams of body weight and the ratio of RNA to DNA for the two investigated physiological periods. Figures 37 and 38 show, likewise as scattergrams, the variations of TPN and TPNH, DPN and DPNH per 100 mgms of mammary tissue (wet weight), and the ratio values of TPNH/TPN and DPN/DPNH. While the scattergrams of the RNA, DNA, and RNA/DNA ratio values suggest monotone increasing functions as the animal progresses through pregnancy and lactation, it should be noted that the other scattergrams represent both increasing and decreasing polynomial functions with, in some cases, marked variations between adjacent time periods.

In order to test for the degree of variation between the corresponding time periods, i.e., P_{i} and/or L_{i}, pair-wise statistical comparisons were determined by the "t" test. The significance levels for the time-period comparisons are given in Table 2. After a brief review of Table 2, it is immediately obvious that most of the periods, in regard to the measured

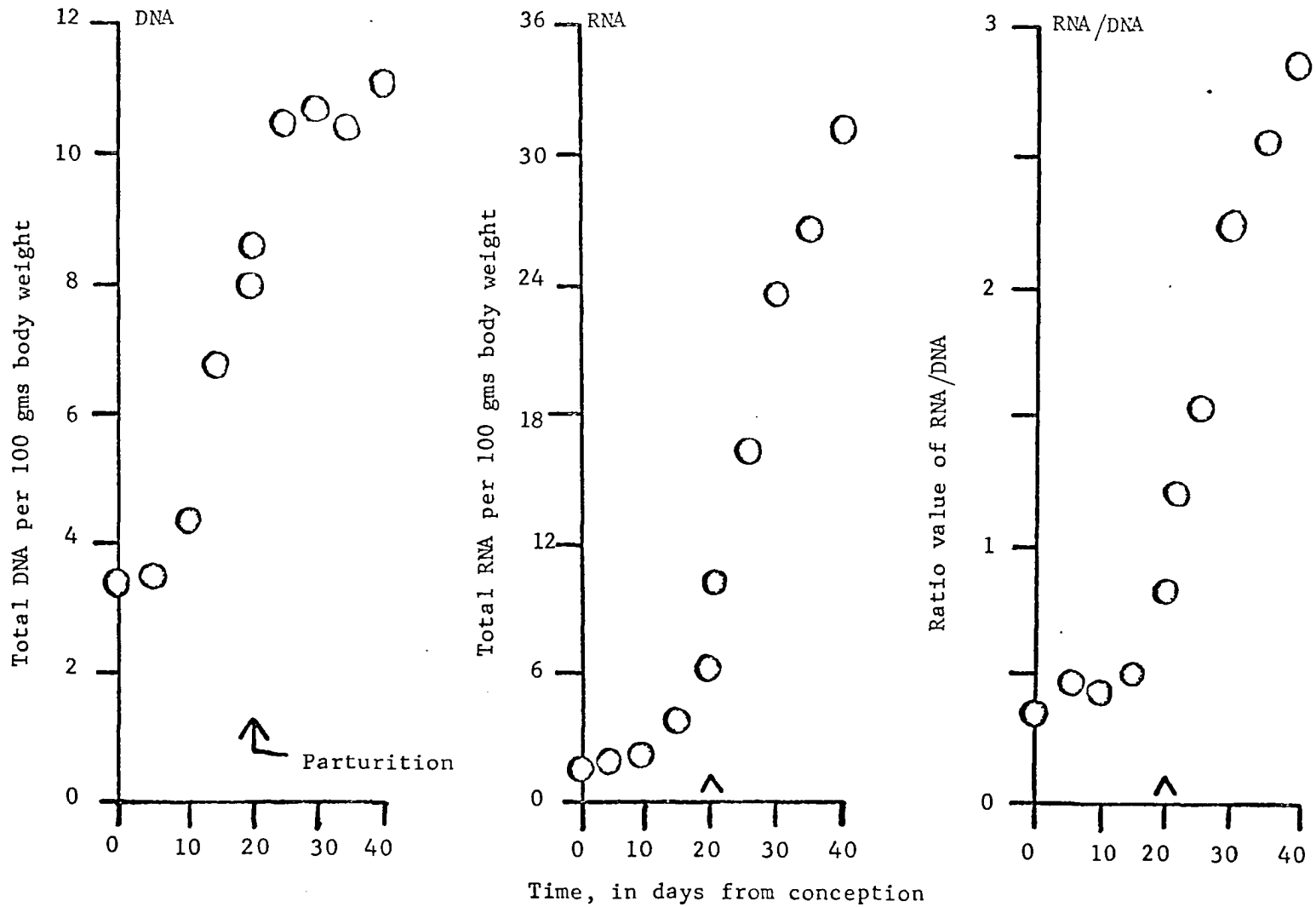

Figure 36. Variation of DNA, RNA and RNA/DNA during pregnancy and lactation

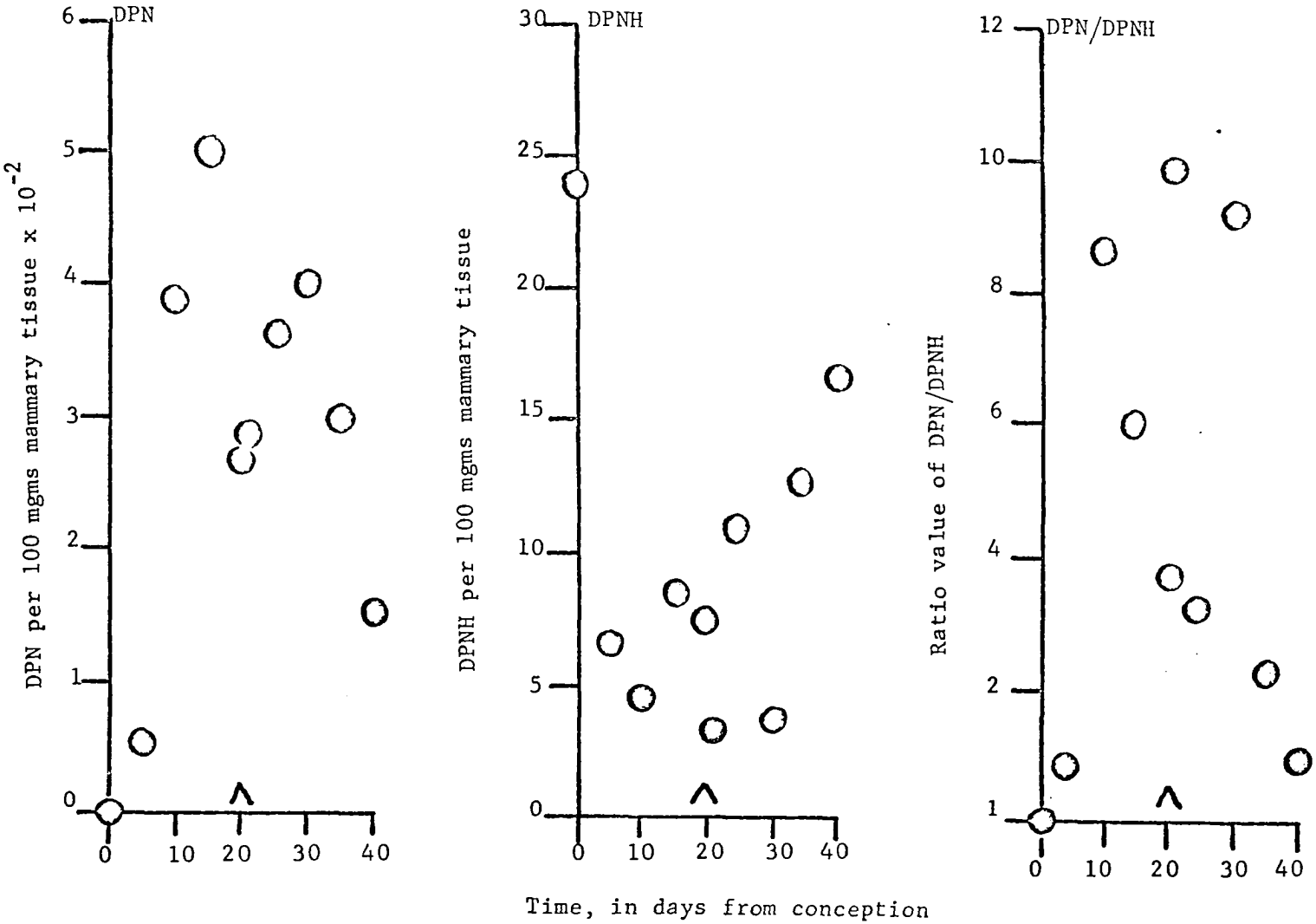

Figure 37. Variation of DPN, DPNH and DPN/DPNH during pregnancy and lactation

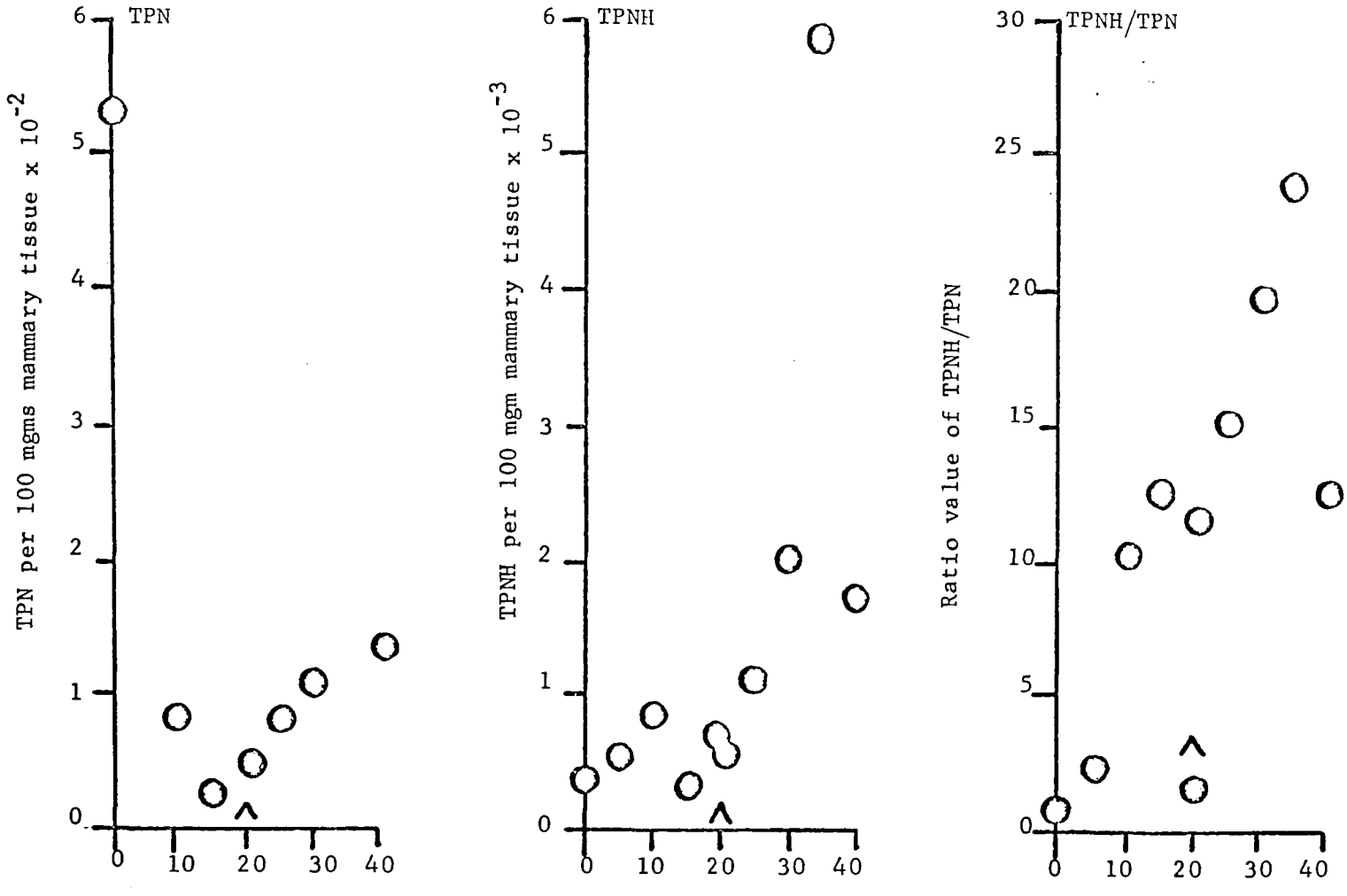

Figure 38. Variation of TPN, TPNH and TPNH/TPN during pregnancy and lactation

Table 2. Significance levels from T-test of paired comparisons for time periods during pregnancy and lactation[a]

Physiological time periods	Physiological time periods									
	N_C	N_{P_5}	$N_{P_{10}}$	$N_{P_{15}}$	$N_{P_{20}}$	N_{L_1}	N_{L_5}	$N_{L_{10}}$	$N_{L_{15}}$	$N_{L_{20}}$
1. RNA and DNA significance levels										
DNA	RNA									
N_C	--	S.5	S.5	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.
N_{P_5}	N.S.	--	N.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.
$N_{P_{10}}$	S.5	S.5	--	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.
$N_{P_{15}}$	H.S.	H.S.	H.S.	--	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.
$N_{P_{20}}$	H.S.	H.S.	H.S.	S.5	--	H.S.	H.S.	H.S.	H.S.	H.S.
N_{L_1}	H.S.	H.S.	H.S.	S.1	N.S.	--	H.S.	H.S.	H.S.	H.S.
N_{L_5}	H.S.	H.S.	H.S.	H.S.	S.1	S.1	--	H.S.	H.S.	H.S.
$N_{L_{10}}$	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	N.S.	--	S.5	H.S.
$N_{L_{15}}$	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	N.S.	N.S.	--	S.1
$N_{L_{20}}$	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	N.S.	N.S.	N.S.	--
2. TPN and TPNH significance levels										
TPNH	TPN									
N_C	--	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.

[a] H.S., highly significant, i.e., $P < 0.001$.
S.1., significant at $P < 0.01$, $P > 0.001$.
S.5, significant at $P < 0.05$, $P > 0.01$.
N.S., non-significant, i.e., $P > 0.05$.
--, no values.

Table 2. (Continued)

Physiological time periods	Physiological time periods									
	N_C	N_{P_5}	$N_{P_{10}}$	$N_{P_{15}}$	$N_{P_{20}}$	N_{L_1}	N_{L_5}	$N_{L_{10}}$	$N_{L_{15}}$	$N_{L_{20}}$
N_{P_5}	H.S.	--	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	N.S.	H.S.
$N_{P_{10}}$	H.S.	H.S.	--	H.S.	H.S.	H.S.	N.S.	N.S.	H.S.	H.S.
$N_{P_{15}}$	S.1	H.S.	H.S.	--	H.S.	S.1	H.S.	H.S.	H.S.	H.S.
$N_{P_{20}}$	H.S.	S.1	S.1	H.S.	--	H.S.	H.S.	H.S.	H.S.	H.S.
N_{L_1}	H.S.	N.S.	H.S.	H.S.	H.S.	--	H.S.	S.1	H.S.	H.S.
N_{L_5}	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	--	N.S.	H.S.	H.S.
$N_{L_{10}}$	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	--	H.S.	N.S.
$N_{L_{15}}$	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	--	H.S.
$N_{L_{20}}$	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	S.1	H.S.	--

3. DPN and DPNH significance levels

DPNH	DPN									
N_C	--	--	--	--	--	--	--	--	--	--
N_{P_5}	H.S.	--	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.
$N_{P_{10}}$	H.S.	N.S.	--	H.S.	H.S.	H.S.	N.S.	N.S.	H.S.	H.S.
$N_{P_{15}}$	S.1	S.5	H.S.	--	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.
$N_{P_{20}}$	S.1	N.S.	S.1	N.S.	--	N.S.	S.1	H.S.	N.S.	H.S.
N_{L_1}	H.S.	S.1	H.S.	H.S.	H.S.	--	S.5	S.1	N.S.	H.S.
N_{L_5}	S.1	H.S.	H.S.	H.S.	H.S.	H.S.	--	N.S.	S.5	H.S.
$N_{L_{10}}$	H.S.	N.S.	N.S.	H.S.	S.1	H.S.	H.S.	--	S.1	H.S.
$N_{L_{15}}$	S.5	S.1	H.S.	S.5	S.5	H.S.	N.S.	H.S.	--	H.S.
$N_{L_{20}}$	N.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	S.5	--

substances, are highly significantly (H.S.) different from each other. However, from Table 2, we must note that N_{P_5} when compared to $N_{P_{10}}$ is the only case of non-significant (or similar) RNA concentrations: N_C versus (-) N_{P_5}, $N_{P_{20}} - N_{L_1}$, $N_{L_5} - N_{L_{10}}$, $N_{L_5} - N_{L_{15}}$, $N_{L_5} - N_{L_{20}}$, $N_{L_{10}} - N_{L_{15}}$, $N_{L_{10}} - N_{L_{20}}$ and $N_{L_{15}} - N_{L_{20}}$ are cases of insignificant DNA concentrations; $N_{P_5} - N_{L_{15}}$, $N_{P_{10}} - N_{L_5}$, $N_{P_{10}} - N_{L_{10}}$, $N_{L_5} - N_{L_{10}}$ and $N_{L_{10}} - N_{L_{20}}$ show similar TPN concentrations; $N_{P_5} - N_{L_1}$ is the only example of similar TPNH concentrations; $N_{P_{10}} - N_{L_5}$, $N_{P_{10}} - N_{L_{10}}$, $N_{P_{20}} - N_{L_1}$, $N_{P_{20}} - N_{L_{15}}$, $N_{L_1} - N_{L_{15}}$ and $N_{L_5} - N_{L_{10}}$ for like (or insignificant) DPN concentrations; and $N_{P_5} - N_{P_{10}}$, $N_{P_5} - N_{P_{20}}$, $N_{P_5} - N_{L_{10}}$, $N_{P_{10}} - N_{L_{10}}$, $N_{P_{15}} - N_{P_{20}}$, and $N_{L_5} - N_{L_{15}}$ for non-significant DPNH concentrations. A summarization of the insignificant time-period groupings, as shown by the mean values and as determined by pair-wise comparisons, is given in Table 3. In this table those means connected by the undermark are insignificant; otherwise significantly different from each other.

B. Artificial Mammogenesis

In order to measure the extent of artificial mammogenesis, reports use the 20th day of normal pregnancy ($N_{P_{20}}$) as the major testing criterion. In accordance with the use of this a priori condition for estimating the extent of artificial mammogenesis, all experimentally ablated and/or

Table 3. Groupings of insignificant means for measured time-periods, pregnancy and lactation

Measured substance	Ranked mean values									
RNA	1.12 N_C	1.68 N_{P_5}	1.86 $N_{P_{10}}$	3.38 $N_{P_{15}}$	6.16 $N_{P_{20}}$	10.28 N_{L_1}	16.23 N_{L_5}	23.67 $N_{L_{10}}$	26.80 $N_{L_{15}}$	31.17 $N_{L_{20}}$
DNA	3.20 N_C	3.42 N_{P_5}	4.32 $N_{P_{10}}$	6.62 $N_{P_{15}}$	7.90 $N_{P_{20}}$	8.49 N_{L_1}	10.47 N_{L_5}	10.59 $N_{L_{10}}$	10.43 $N_{L_{15}}$	11.07 $N_{L_{20}}$
TPN	24.6 $N_{P_{15}}$	43.6 N_{L_1}	76.4 N_{L_5}	80.6 $N_{P_{10}}$	104.8 $N_{L_{10}}$	135.9 $N_{L_{20}}$	247.4 $N_{L_{15}}$	277.1 N_{P_5}	406.1 $N_{P_{20}}$	525.8 N_C
TPNH	298.4 $N_{P_{15}}$	373.3 N_C	557.6 N_{L_1}	559.7 N_{P_5}	678.2 $N_{P_{20}}$	851.1 $N_{P_{10}}$	1146.0 N_{L_5}	1700.1 $N_{L_{20}}$	2002.7 $N_{L_{10}}$	5851.0 $N_{L_{15}}$
DPN	0.0 N_C	51.1 N_{P_5}	154.1 $N_{L_{20}}$	269.1 $N_{P_{20}}$	285.7 N_{L_1}	292.0 $N_{L_{15}}$	359.2 N_{L_5}	387.4 $N_{P_{10}}$	397.4 $N_{P_{10}}$	496.9 $N_{P_{15}}$
DPNH	2.9 N_{L_1}	4.2 $N_{L_{10}}$	4.5 $N_{P_{10}}$	6.1 N_{P_5}	7.3 $N_{P_{20}}$	8.3 $N_{P_{15}}$	11.1 N_{L_5}	12.8 $N_{L_{15}}$	16.8 $N_{L_{20}}$	24.6 N_C

hormonally injected animals were compared to the proper $N_{P_{20}}$ value.

To test the extent of artificial mammogenesis, the experimental animals were stratified into two different groupings, i) by operative group regardless of treatment group, i.e., all animals being in the same operative state (Charts 2 through 8), and ii) by treatment group regardless of operative group, i.e., all experimental animals receiving the same hormonal injection; see Table 4. This stratification was done in the attempt to extract more information from the experimentation. The stratified groups were statistically tested by Dunnett's test (166), chosen for the following three reasons:

i) Analysis of the experimental data by the appropriate analysis of variance model, not shown herein, showed a high degree of variation within treatment groups, operative groups and their interaction; this suggested a comparison of means was necessary to gain insight into individual treatment and/or surgical difference.

ii) Analysis of the experimental data by paired comparisons, i.e., multiple "t" tests, would be in appropriate due to an insufficient number of total degrees of freedom, i.e., the number of needed comparisons exceeded the total available degrees of freedom.

iii) Analysis of the data by a multiple range test, such as the Duncan test, would give unnecessary comparisons--since the literature uses $N_{P_{20}}$ as an <u>a priori</u> criterion, and would be inappropriate due to the insufficient number of degrees of freedom.

The mean values of the operative groups regardless of treatment group and the pooled variances necessary for the Dunnett test procedure are shown in Tables 4 and 5, respectively. Table 5 also gives the 95% and 99% confidence intervals of the control $N_{P_{20}}$, for each operative-treatment group. Related figures give a graphic representation of the results from the Dunnett test, and Table 6 gives a summary of the data represented in these figures.

1. RNA comparisons

Upon inspection of the results from the Dunnett test as shown in Figures 39 and 40, it is immediately apparent that $O_1 - T_4$ is the only operative-treatment combination group that has RNA values insignificantly different from $N_{P_{20}}$. However, if one studies Figures 39 and 40, along with Table 6, one will notice that,

a. O_3 without hormonal therapy tended to increase the RNA concentration of the mammary gland, but was less than the RNA concentration at $N_{P_{20}}$.

b. O_2 and/or O_3 with hormonal therapy usually tended to increase the RNA concentration of the gland above that of $N_{P_{20}}$.

c. O_1 with hormonal therapy tended to increase the RNA concentration such that it was approximately equivalent to $N_{P_{20}}$.

d. Hypophysectomy tended to lower the RNA concentration of the gland--regardless of additional surgery, i.e., O_4, O_5, O_6 and O_7--without hormonal therapy or with hormonal therapy when STH and/or M were excluded from the therapy from that of $N_{P_{20}}$; however, when STH and/or M were included in the therapy, the

Table 4. Means by operative group within treatment group

Treatment group	Operative group	Number of experimental animals	Measured substance					
			RNA	DNA	TPN	TPNH	DPN	DPNH
T_1	O_1	12	1.12	3.20	525.8	373.3	0.0	24.6
	O_1^2	6	1.20	3.12	432.0	155.5	70.7	8.4
	O_2^3	6	4.06	3.95	176.8	47.7	6.5	0.0
	O_3^4	6	1.65	1.96	761.7	236.7	15.7	0.0
	O_4^5	6	1.39	1.66	637.7	223.2	19.5	5.1
	O_5^6	6	1.29	1.51	629.5	163.7	15.1	1.7
	O_7	6	1.45	2.05	765.8	265.6	0.0	39.7
T_2	O_1	8	3.87	6.00	317.1	301.2	215.2	3.1
	O_1^2	19	4.78	7.24	200.5	192.5	15.6	1.5
	O_2^3	8	4.61	7.33	192.8	79.0	14.9	6.7
	O_3^4	8	1.36	2.05	465.6	451.6	0.0	27.6
	O_4^5	8	1.36	1.72	816.3	375.5	36.1	9.2
	O_5^7	8	1.11	1.90	775.6	219.6	0.0	4.4
T_3	O_1	8	8.71	7.88	328.1	275.6	290.2	3.4
	O_1^2	17	15.40	9.40	231.8	259.6	19.8	9.4
	O_2^4	8	0.87	1.87	502.5	356.8	29.6	12.7
	O_4^5	8	1.58	1.65	605.6	190.5	29.5	7.6
T_4	O_1	8	6.11	5.65	209.5	282.8	91.0	0.0
	O_1^2	19	10.31	6.77	248.5	226.1	0.0	14.4
	O_2^3	15	11.65	7.17	310.7	183.3	0.0	11.3
	O_7	8	1.05	1.55	975.9	351.3	28.9	7.0
T_5	O_1	8	6.87	5.97	399.0	367.1	145.8	0.0
	O_1^3	8	14.62	8.75	254.5	193.4	21.6	49.6
	O_3^5	8	0.77	1.45	427.6	188.1	31.7	12.9
	O_5^7	8	0.63	1.67	756.0	310.0	39.9	11.4
T_6	O_1	8	9.30	6.66	215.6	247.9	307.5	3.7
	O_1^4	8	2.79	2.85	229.7	296.3	29.5	6.7
	O_4^5	9	2.39	2.42	316.5	364.0	31.6	5.7
T_7	O_2	8	15.65	8.42	236.7	416.6	15.9	7.8
T_8	O_7	8	3.69	3.54	559.7	442.2	25.4	3.2
T_9	O_7	8	6.01	6.03	427.6	517.4	19.6	2.9
T_{10}	O_7	18	2.76	2.80	152.5	325.7	14.8	2.6

Table 5. Pooled variances and confidence intervals from Dunnett's procedure

Comparison ($N_{P_{20}}$ verses)	Degrees of freedom	(1) p value	(2) Pertinent statistic	Measured substance					
				RNA	DNA	TPN	TPNH	DPN	DPNH
T_1	48	7	s_p	0.29	0.24	20.06	14.14	3.55	1.00
			95% C.I.	0.45	0.36	29.91	21.09	5.30	1.48
			99% C.I.	0.54	0.44	36.26	25.55	6.42	1.79
T_2	60	6	s_p	0.38	0.38	20.79	17.90	4.74	0.87
			95% C.I.	0.59	0.59	32.40	27.89	7.40	1.35
			99% C.I.	0.72	0.72	39.36	33.88	8.99	1.64
T_3	44	4	s_p	0.39	0.32	19.48	18.14	7.90	0.90
			95% C.I.	0.72	0.59	45.67	32.98	14.37	1.64
			99% C.I.	0.90	0.74	56.86	41.06	17.89	2.05
T_4	53	4	s_p	0.30	0.32	17.74	17.33	4.83	0.80
			95% C.I.	0.54	0.59	32.10	31.36	8.76	1.46
			99% C.I.	0.67	0.73	39.73	38.83	10.84	1.81
T_5	35	4	s_p	0.38	0.34	18.69	16.85	7.27	1.24
			95% C.I.	0.70	0.62	34.37	30.97	13.36	2.29
			99% C.I.	0.88	0.78	42.96	38.71	16.70	2.86
T_6	29	3	s_p	0.39	0.33	15.50	21.01	9.65	0.48
			95% C.I.	0.80	0.65	31.65	42.88	19.70	0.98
			99% C.I.	1.01	0.82	40.13	54.37	24.98	1.24
T_7	14	1	s_p	0.46	0.34	14.05	20.05	9.03	0.76
			95% C.I.	0.14	0.11	4.26	6.08	2.74	0.24
			99% C.I.	0.21	0.15	5.93	8.46	3.81	0.33

Table 5. (Continued)

Comparison ($N_{P_{20}}$ verses)	Degrees of freedom	(1) p value	(2) Pertinent statistic	Measured substance RNA	DNA	TPN	TPNH	DPN	DPNH
T_8	14	1	s_p	0.40	0.30	21.28	39.14	10.12	0.64
			95% C.I.	0.13	0.09	6.44	11.83	3.06	0.19
			99% C.I.	0.18	0.12	8.97	16.48	4.26	0.27
T_9	14	1	s_p	0.34	0.28	17.54	25.34	9.52	0.48
			95% C.I.	0.11	0.09	5.61	7.66	2.89	0.15
			99% C.I.	0.15	0.12	7.81	10.67	4.02	0.21
T_{10}	24	1	s_p	0.34	0.32	15.52	22.30	8.78	0.62
			95% C.I.	0.10	0.10	4.51	6.49	2.55	0.19
			99% C.I.	0.14	0.04	6.13	8.82	3.46	0.25
O_1	53	6	s_p	0.36	0.33	16.17	16.05	11.14	1.06
			95% C.I.	0.57	0.52	25.38	25.21	17.49	1.66
			99% C.I.	0.70	0.63	30.89	30.68	21.28	2.02
O_2	71	5	s_p	0.36	0.32	15.12	19.73	5.32	0.72
			95% C.I.	0.60	0.54	25.14	32.82	8.97	1.21
			99% C.I.	0.74	0.62	30.69	40.06	10.79	1.48
O_3	40	4	s_p	0.33	0.37	15.40	9.73	3.95	0.93
			95% C.I.	0.59	0.67	28.10	17.75	7.20	1.70
			99% C.I.	0.74	0.83	34.96	22.08	8.96	2.12
O_4	33	4	s_p	0.38	0.36	15.87	21.26	6.13	1.52
			95% C.I.	0.68	0.65	29.28	39.23	11.30	2.79
			99% C.I.	0.85	0.82	36.58	50.00	14.12	3.49

Table 5. (Continued)

Comparison ($N_{P_{20}}$ verses)	Degrees of freedom	(1) p value	(2) Pertinent statistic	Measured substance RNA	DNA	TPN	TPNH	DPN	DPNH
O_5	41	5	s_p	0.40	0.31	24.69	20.24	5.85	0.37
			95% C.I.	0.67	0.53	41.68	34.18	9.88	0.61
			99% C.I.	0.82	0.66	51.36	42.11	12.17	0.76
O_6	12	1	s_p	0.30	0.30	20.98	16.56	8.57	0.38
			95% C.I.	0.09	0.09	6.47	5.10	2.64	0.13
			99% C.I.	0.12	0.12	9.06	7.14	3.69	0.18
O_7	63	7	s_p	0.27	0.24	22.70	18.82	3.22	0.77
			95% C.I.	0.39	0.36	33.48	27.77	4.75	1.13
			99% C.I.	0.47	0.43	40.39	33.50	5.73	1.37

Notes:

(1) p-value is the number of treatment means being compared, excluding the control.

(2) s_p is the pooled standard error of the combined treatment and control groups.
95% C.I. is the 95 percent confidence interval of the control mean, $\pm$.
99% C.I. is the 99 percent confidence interval of the control mean, $\pm$.

Table 6. Summarization of results from Dunnett test procedure

(1) Primary strata		(2) C.I. of control ($N_{P_{20}}$)		Results of Dunnett comparison ($N_{P_{20}}$ verses)		
(T_i)	(O_i)	95%	99%	Non-significantly different groups	Significantly different groups: Group means less	Significantly different groups: Group means greater
		1. RNA ($N_{P_{20}}$ = 6.16 mgms Total RNA per 100 gms body weight)				
T_1		5.71-6.61	5.62-6.70		all	
T_2		5.56-6.75	5.44-6.88		all	
T_3		5.44-6.88	5.26-7.06		O_4 & O_5	O_1,O_2 & O_3
T_4		5.62-6.70	5.49-6.83	O_1	O_7	O_2 & O_3
T_5		5.46-6.86	5.28-7.04		O_1,O_5 & O_7	O_3
T_6		5.36-6.96	5.15-7.17		O_4 & O_5	O_1
T_7		6.02-6.30	5.95-6.37		O_2	
T_8		6.03-6.29	5.98-6.34		O_7	
T_9		6.05-6.27	6.01-6.31		O_7	
T_{10}		6.06-6.26	6.02-6.30		O_7	
	O_1	5.59-6.73	5.46-6.86	T_4	T_1 & T_2	T_3,T_5 & T_6
	O_2	5.56-6.76	5.42-6.90		T_1 & T_2	T_3,T_4 & T_7
	O_3	5.57-6.75	5.42-6.90		T_1 & T_2	T_4 & T_5
	O_4	5.48-6.84	5.31-7.01		all	
	O_5	5.49-6.83	5.34-6.98		all	
	O_6	6.07-6.25	6.04-6.28		T_1	
	O_7	5.77-6.55	5.69-6.63	T_9	all others	
		2. DNA ($N_{P_{20}}$ = 7.90 mgms Total DNA per 100 gms body weight)				
T_1		7.54-8.26	7.46-8.34		all	
T_2		7.31-8.49	7.18-8.62	O_3	all others	
T_3		7.31-8.49	7.16-8.64	O_1	O_4 & O_5	O_2
T_4		7.31-8.49	7.17-8.63		all others	
T_5		7.28-8.52	7.12-8.68		O_1,O_5 & O_7	O_3
T_6		7.25-8.55	7.08-8.72		all	

Table 6. (Continued)

(1) Primary strata (T_i)	(O_i)	(2) C.I. of control ($N_{P_{20}}$) 95%	99%	Results of Dunnett comparison ($N_{P_{20}}$ verses) Non-significantly different groups	Significantly different groups: Group means less	Group means greater
T_7		7.79-8.01	7.75-8.05			O_2
T_8		7.81-7.99	7.78-8.02		O_7	
T_9		7.81-7.99	7.78-8.02		O_7	
T_{10}		7.80-8.00	7.76-8.04		O_7	
	O_1	7.38-8.42	7.27-8.52	T_3	all others	
	O_2	7.36-8.44	7.25-8.55	T_7	T_1,T_2 & T_4	T_3
	O_3	7.22-8.57	7.07-8.73	T_2	T_1 & T_4	T_5
	O_4	7.25-8.55	7.08-8.72		all	
	O_5	7.37-8.43	7.24-8.56		all	
	O_6	7.81-7.99	7.78-8.02		T_1	
	O_7	7.54-8.26	7.47-8.33		all	
		3. TPN ($N_{P_{20}}$ = 406.1 μM per 100 mgms mammary tissue)				
T_1		376.2-436.0	369.8-442.4	O_2	O_3	all others
T_2		373.7-428.5	366.7-445.5		O_1,O_2 & O_3	O_4,O_5 & O_7
T_3		360.4-451.8	349.2-463.0		O_1 & O_2	O_4 & O_5
T_4		374.0-437.2	366.4-445.8		O_1,O_2 & O_3	O_7
T_5		371.7-440.5	363.1-449.1	O_1 & O_5	O_3	O_7
T_6		374.5-437.7	366.0-446.2		all	
T_7		401.8-410.4	400.2-412.0		O_2	
T_8		399.7-412.5	397.1-415.1			O_7
T_9		400.5-411.7	398.3-413.9			O_7
T_{10}		401.6-410.6	400.0-412.2		O_7	
	O_1	380.7-431.5	375.2-437.0	T_5	T_2,T_3,T_4 & T_6	T_1
	O_2	381.0-431.2	375.4-436.8		T_2,T_3,T_4 & T_7	T_1
	O_3	378.0-434.2	371.1-441.1		all	
	O_4	376.8-435.4	369.5-442.7		T_6	T_1,T_2 & T_3
	O_5	364.4-447.8	354.7-456.5	T_5	T_6	T_1,T_2 & T_3

Table 6. (Continued)

(1) Primary strata (T_i)	(O_i)	(2) C.I. of control ($N_{P_{20}}$) 95%	99%	Results of Dunnett comparison ($N_{P_{20}}$ verses) Non-significantly different groups	Significantly different groups: Group means less	Group means greater
	O_6	399.6-412.6	397.0-415.2			T_1
	O_7	372.6-439.6	365.7-446.5	T_9	T_{10}	T_1,T_2,T_4,T_5 & T_8
		4. TPNH ($N_{P_{20}}$ = 678.2 μM per 100 mgms mammary tissue)				
T_1		657.1-699.3	652.6-703.8		all	
T_2		650.3-706.1	644.3-712.1		all	
T_3		645.2-711.2	637.1-719.3		all	
T_4		646.8-709.6	639.4-717.0		all	
T_5		647.2-709.2	639.5-716.9		all	
T_6		635.3-721.1	623.8-732.6		all	
T_7		672.1-684.3	669.7-686.7		all	
T_8		666.4-690.0	661.7-694.7		all	
T_9		679.5-685.9	667.5-688.9		all	
T_{10}		671.7-684.7	669.4-687.0		all	
	O_1	653.0-703.4	647.5-708.9		all	
	O_2	645.4-711.0	638.1-718.3		all	
	O_3	660.6-695.8	656.1-700.3		all	
	O_4	639.0-717.4	628.2-728.3		all	
	O_5	644.0-712.4	636.1-720.3		all	
	O_6	673.1-683.3	671.1-685.3		all	
	O_7	650.4-706.0	644.7-711.7		all	
		5. DPN ($N_{P_{20}}$ = 269.1 μM per 100 mgms mammary tissue)				
T_1		263.8-274.4	262.7-275.5		all	
T_2		261.7-276.5	260.1-278.1		all	
T_3		254.7-283.5	251.2-287.0		O_2,O_4 & O_5	O_1

Table 6. (Continued)

(1) Primary strata (T_i)	(O_i)	(2) C.I. of control ($N_{P_{20}}$) 95%	99%	Results of Dunnett comparison ($N_{P_{20}}$ verses) Non-significantly different groups	Significantly different groups: Group means less	Group means greater
T_4		260.3-277.9	258.3-279.9		all	
T_5		255.7-282.5	252.4-285.8		all	
T_6		249.4-288.8	244.1-294.1		O_4 & O_5	O_1
T_7		266.4-271.8	265.3-272.9		O_2	
T_8		266.0-272.2	264.8-273.4		O_7	
T_9		266.2-272.0	265.1-273.1		O_7	
T_{10}		266.5-271.7	265.6-272.6		O_7	
	O_1	251.6-286.6	247.8-290.4		T_1,T_2,T_4 & T_5	T_3 & T_6
	O_2	260.1-278.1	258.3-279.9		all	
	O_3	261.9-276.3	260.1-278.1		all	
	O_4	257.8-280.4	255.0-283.2		all	
	O_5	259.2-279.0	256.9-281.3		all	
	O_6	266.5-271.7	265.4-272.8		all	
	O_7	264.3-273.9	263.4-274.8		all	
6. DPNH ($N_{P_{20}}$ = 7.3 μM per 100 mgms mammary tissue)						
T_1		5.8-8.8	5.5-9.1	O_2	O_3,O_4,O_5 & O_6	O_1 & O_7
T_2		5.9-8.7	5.7-8.9	O_3	O_1,O_2 & O_7	O_4 & O_5
T_3		5.7-8.9	5.2-9.4	O_5	O_1	O_2 & O_4
T_4		5.8-8.8	5.5-9.1	O_7	O_1	O_2 & O_4
T_5		5.0-9.6	4.4-10.2		O_1	O_3,O_5 & O_7
T_6		6.3-8.3	6.1-8.5	O_4	O_1 & O_5	
T_7		7.1-7.5	7.0-7.6	O_2		
T_8		7.1-7.5	7.0-7.6		O_7	
T_9		7.1-7.5	7.1-7.5		O_7	
T_{10}		7.1-7.5	7.1-7.5		O_7	

Table 6. (Continued)

(1) Primary strata		(2) C.I. of control ($N_{P_{20}}$)		Results of Dunnett comparison ($N_{P_{20}}$ verses)		
(T_i)	(O_i)	95%	99%	Non-significantly different groups	Significantly different groups: Group means less	Significantly different groups: Group means greater
	O_1	5.6-9.0	5.3-9.3		T_2,T_3,T_4,T_5 & T_6	T_1
	O_2	6.1-8.5	5.8-8.8	T_1 & T_7	T_2	T_3 & T_4
	O_3	5.6-9.0	5.2-9.4	T_2	T_1	T_4 & T_5
	O_4	4.5-10.1	3.8-10.8	T_6	T_1	T_2 & T_3
	O_5	6.7-7.9	6.5-8.1	T_3	T_1 & T_6	T_2 & T_5
	O_6	7.2-7.4	7.1-7.5		T_1	
	O_7	6.2-8.4	5.9-8.7	T_4	T_2,T_8,T_9 & T_{10}	T_1,T_5

Notes:

(1) T_i = Treatment groups regardless of operative group,
O_i = Operative group regardless of treatment group.

(2) Confidence intervals associated with $N_{P_{20}}$ as determined by the Dunnett procedure (see Table 5).

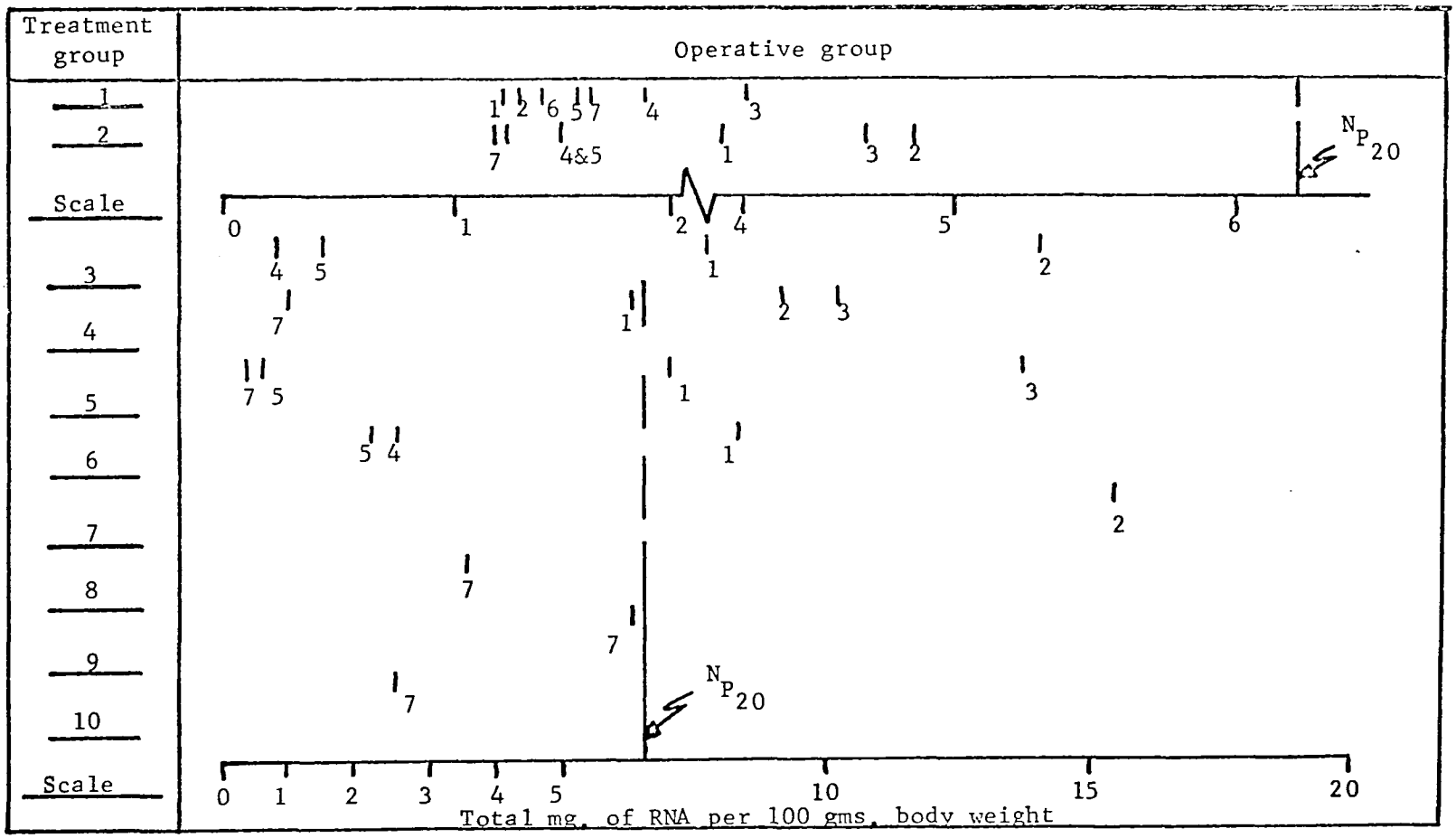

Figure 39. Graphic representation of RNA results from Dunnett's test, stratified by treatment group

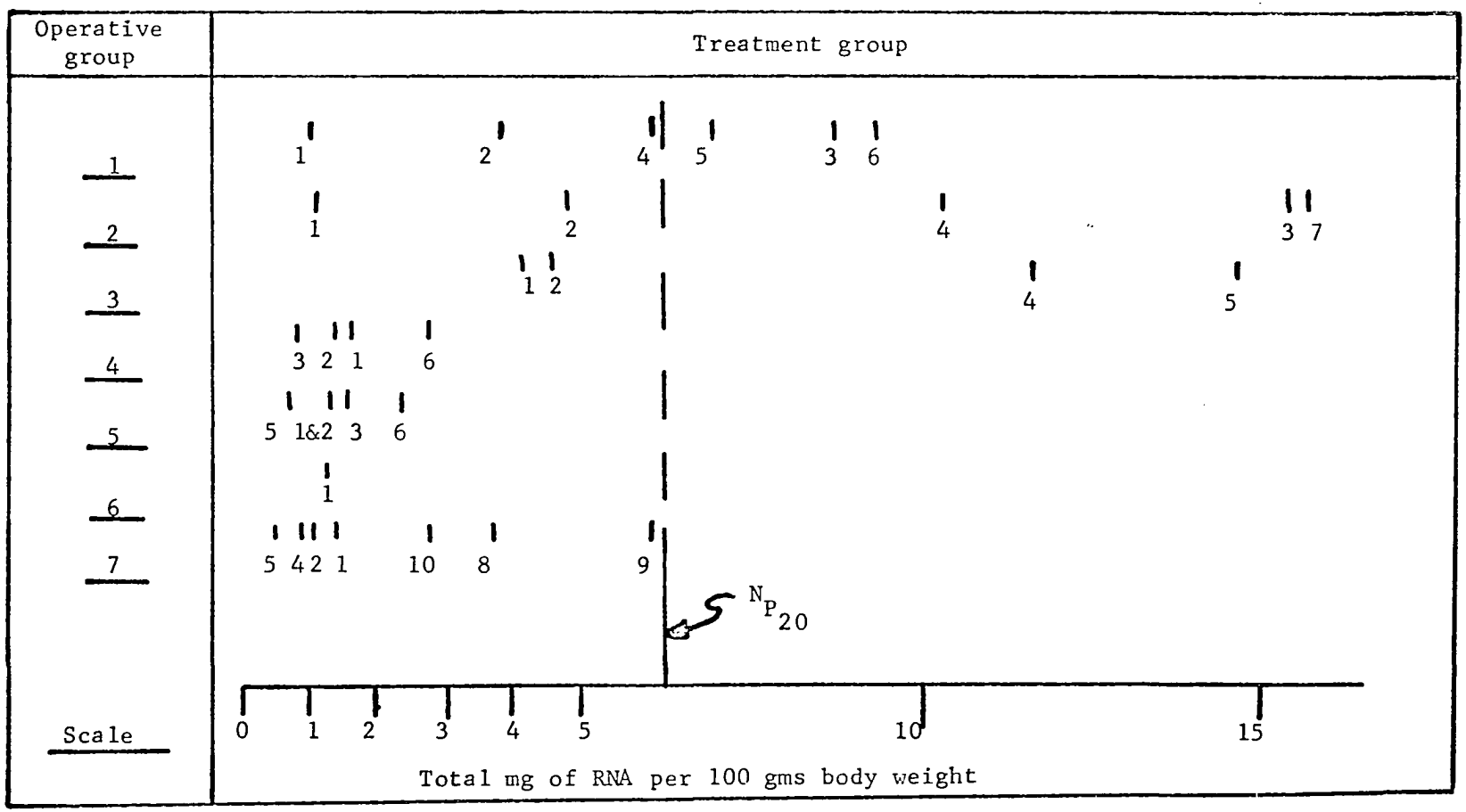

Figure 40. Graphic representation of RNA results from Dunnett's test, stratified by operative group

RNA concentration tended to increase towards that of $N_{P_{20}}$, with exerting a greater effect than STH.

e. Any hormonal therapy tended to increase the total RNA in the mammary gland per 100 gms. body weight of animal for O_1, O_2 and O_3, such that the RNA concentration was equivalent to or greater than $N_{P_{20}}$.

f. All hormonal therapy tended to be ineffectual after hyposectomy--with or without additional surgery, with the exception of STH and M; M tended to increase the RNA concentration to that of $N_{P_{20}}$.

2. DNA concentration

It is apparent from observation of Figures 41 and 42, and Table 6 that O_1 - T_3, O_2 - T_7 and O_3 - T_2 are the only operative-treatment combinations that have DNA values statistically similar to $N_{P_{20}}$. Additional information found in the above mentioned figures and table is as follows:

a. O_1, with and without hormonal therapy, tended to yield a DNA concentration lower than or equivalent to $N_{P_{20}}$.

b. O_2, with hormonal therapy, tended to produce a DNA concentration equivalent to or greater than that at $N_{P_{20}}$.

c. O_2, without replacement therapy, tended to have a DNA value lower than that of $N_{P_{20}}$.

d. O_3 tended to have a variable DNA concentration when compared with $N_{P_{20}}$, according to the therapy given.

e. O_4, with or without additional surgery, tended to be lower

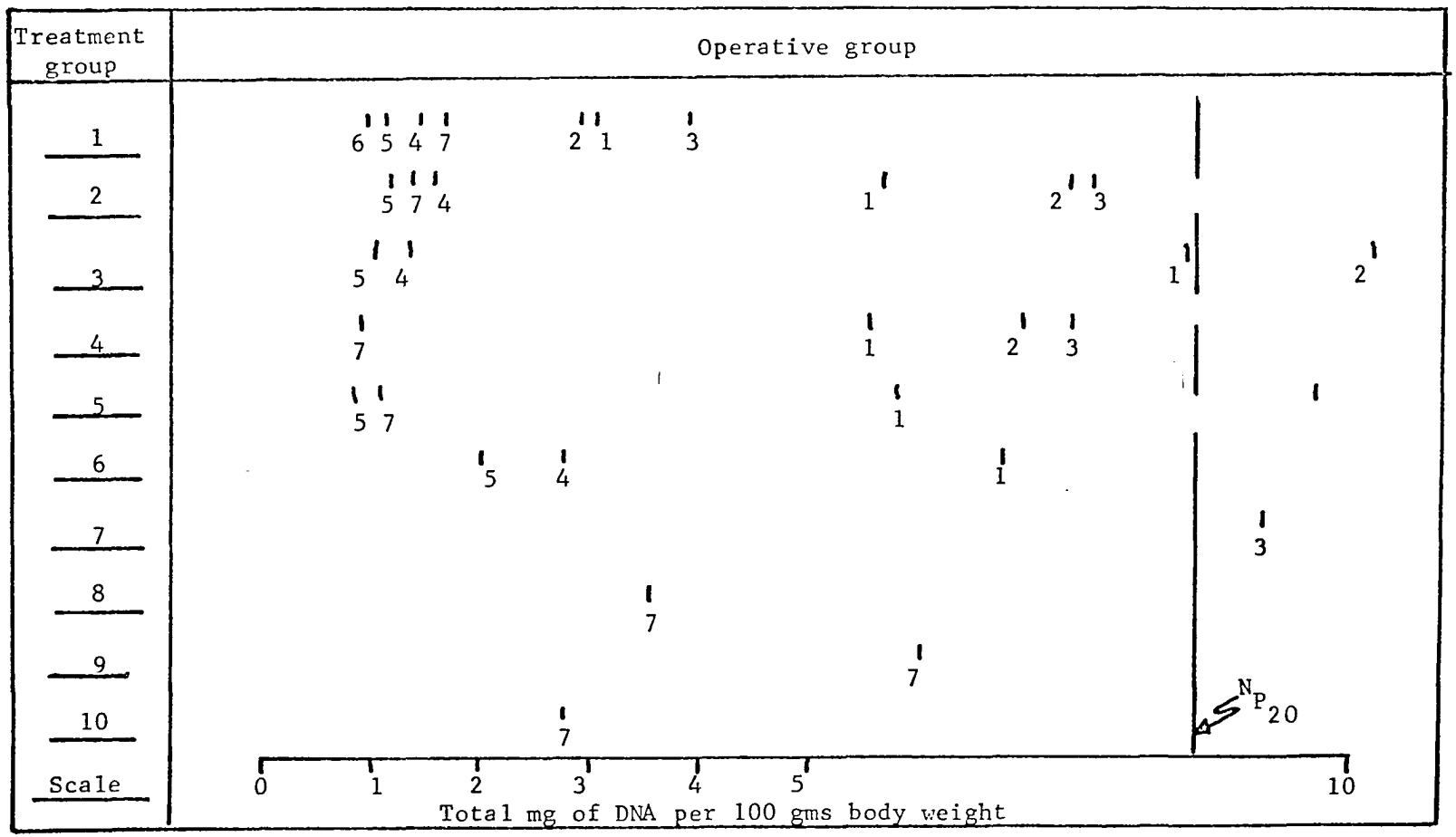

Figure 41. Graphic representation of DNA results from Dunnett's test, stratified by treatment group

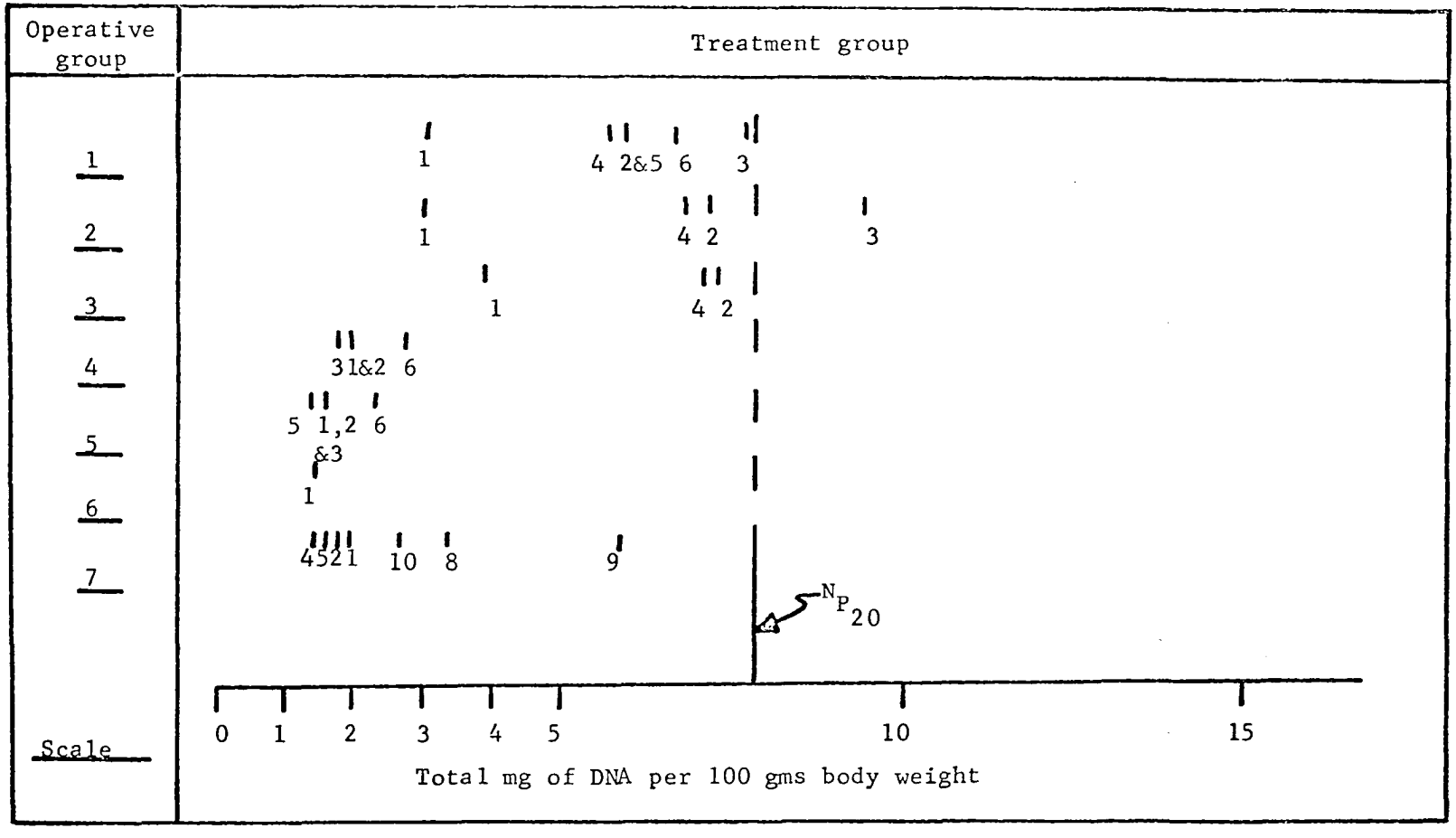

Figure 42. Graphic representation of DNA results from Dunnett's test, stratified by operative group

than $N_{P_{20}}$.

f. Any hormonal therapy tended to increase the DNA concentration for O_1, O_2, and O_3, but tended to have little or no effect on the DNA concentration for O_4--regardless of secondary surgery.

3. TPN concentration

Inspection of Table 6, Figure 43 and Figure 44, shows that $O_1 - T_5$, $O_2 - T_1$, $O_5 - T_5$ and $O_7 - T_9$ give TPN values statistically analogous to $N_{P_{20}}$. Trend effects are as follows:

a. O_1, without therapy, tended to produce a TPN concentration greater than $N_{P_{20}}$; with therapy, tended to be lower than or equivalent to the TPN concentration at $N_{P_{20}}$.

b. O_2, without hormonal replacement, like O_1, tended to have a higher TPN value than $N_{P_{20}}$; with therapy, the TPN values were in all cases lower than the TPN value at $N_{P_{20}}$.

c. O_3, with or without hormonal therapy, tended to be lower than the TPN value at $N_{P_{20}}$.

d. Hypophysectomy did not seem to offer the primary effect on TPN as it did with RNA and DNA, producing variable, i.e., low and high, TPN values when compared to $N_{P_{20}}$.

e. T_1 in all cases except O_3 tended to increase TPN values above that at $N_{P_{20}}$.

f. T_2 and T_3 tended to decrease the TPN concentration below that at $N_{P_{20}}$ with O_1, O_2 and O_3; however, these treatments tended to increase the TPN values above that of $N_{P_{20}}$ when hypophysectomy--regardless of additional surgery--was performed.

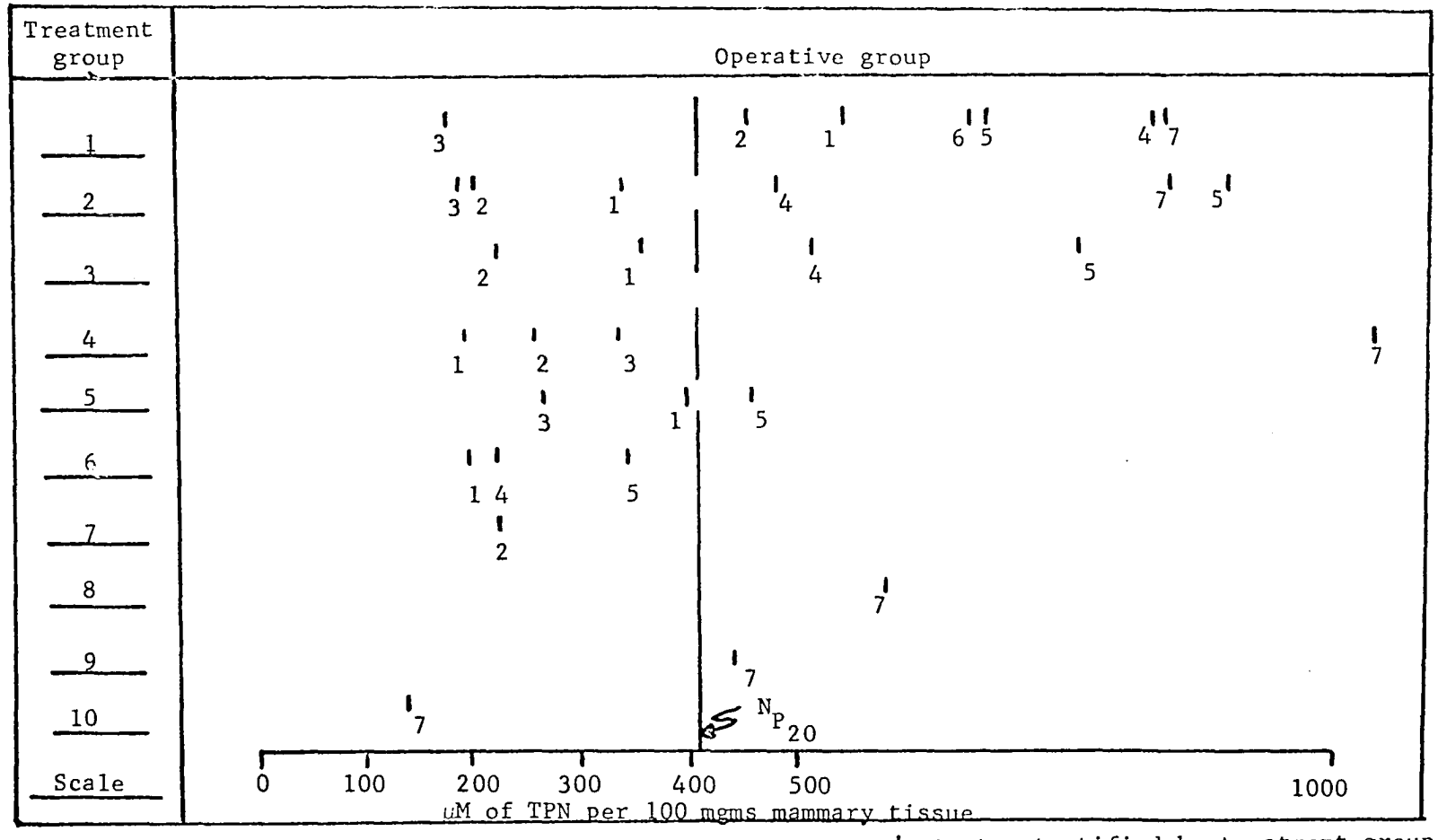

Figure 43. Graphic representation of TPN results from Dunnett's test, stratified by treatment group

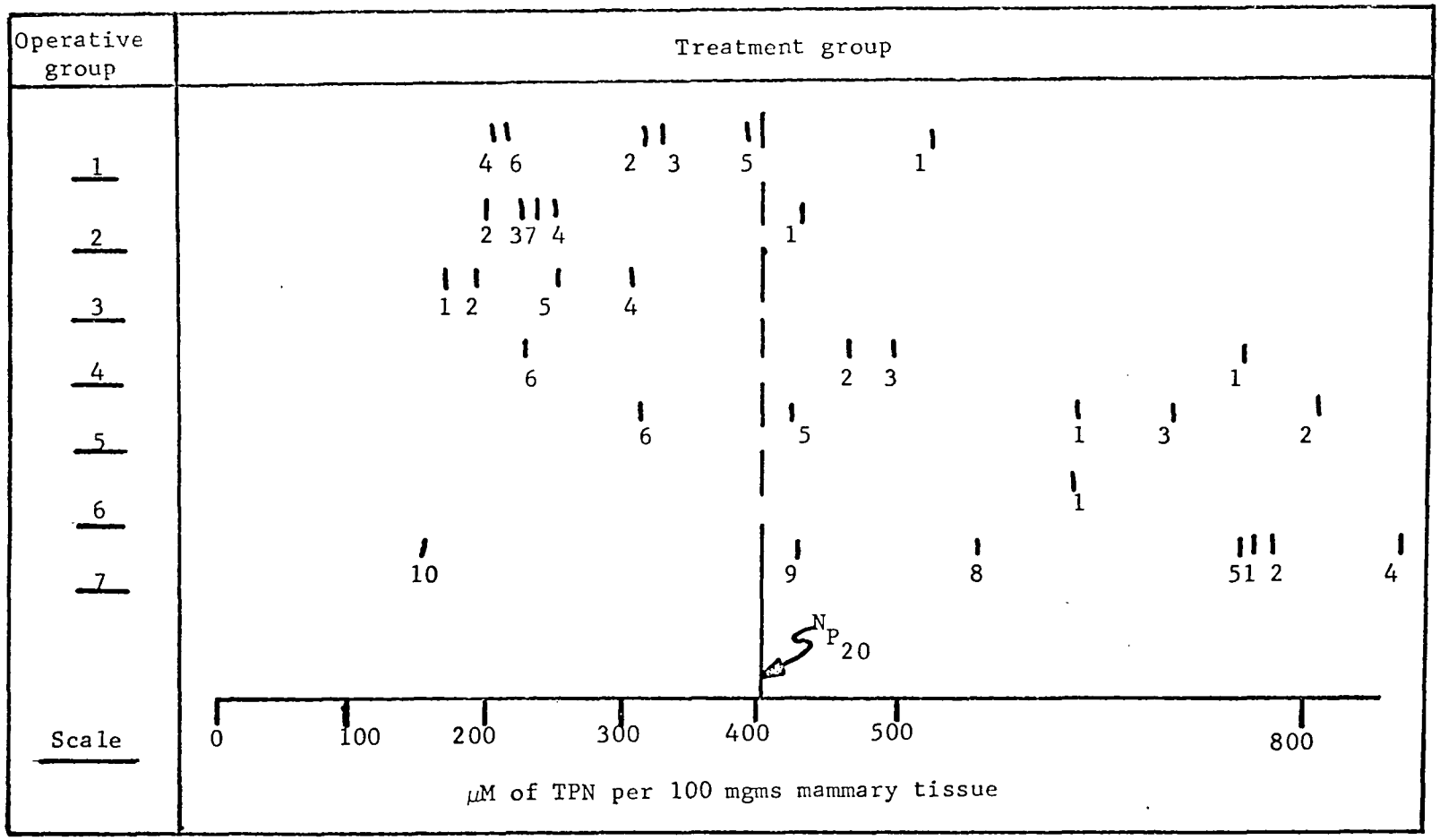

Figure 44. Graphic representation of TPN results from Dunnett's test, stratified by operative group

g. Hypophysectomy, where hormonal therapy excluded M, tended to produce higher TPN values than those estimated at $N_{P_{20}}$; therapy including M, tended to produce values lower than or equivalent values to those for $N_{P_{20}}$.

4. TPNH concentration

Upon examination of Figure 45 and 46, one immediately finds no operative-treatment combination statistically equivalent in TPNH concentration to that of $N_{P_{20}}$. Table 6 substantiates the results and indicates that all operative-treatment combinations produced TPNH values lower than those at $N_{P_{20}}$.

5. DPN concentration

The results of the Dunnett test as presented in Table 6 and Figures 47 and 48, again show that the DPN values from all operative-treatment combinations are significantly different from those obtained at $N_{P_{20}}$. However, $O_1 - T_3$ and $O_1 - T_6$ tended to be higher in DPN concentration as opposed to lower for all other operative-treatment combinations when compared to $N_{P_{20}}$.

6. DPNH concentration

Investigation of the results found in Figures 49 and 50, along with Table 6, shows that $O_2 - T_1$, $O_2 - T_7$, $O_3 - T_2$, $O_4 - T_6$, $O_5 - T_3$ and $O_7 - T_4$ are cases of DPNH similarity with the results of $N_{P_{20}}$. The results from the various operative-treatment combinations were quite variable, and gave no insight into treatment and/or operative group

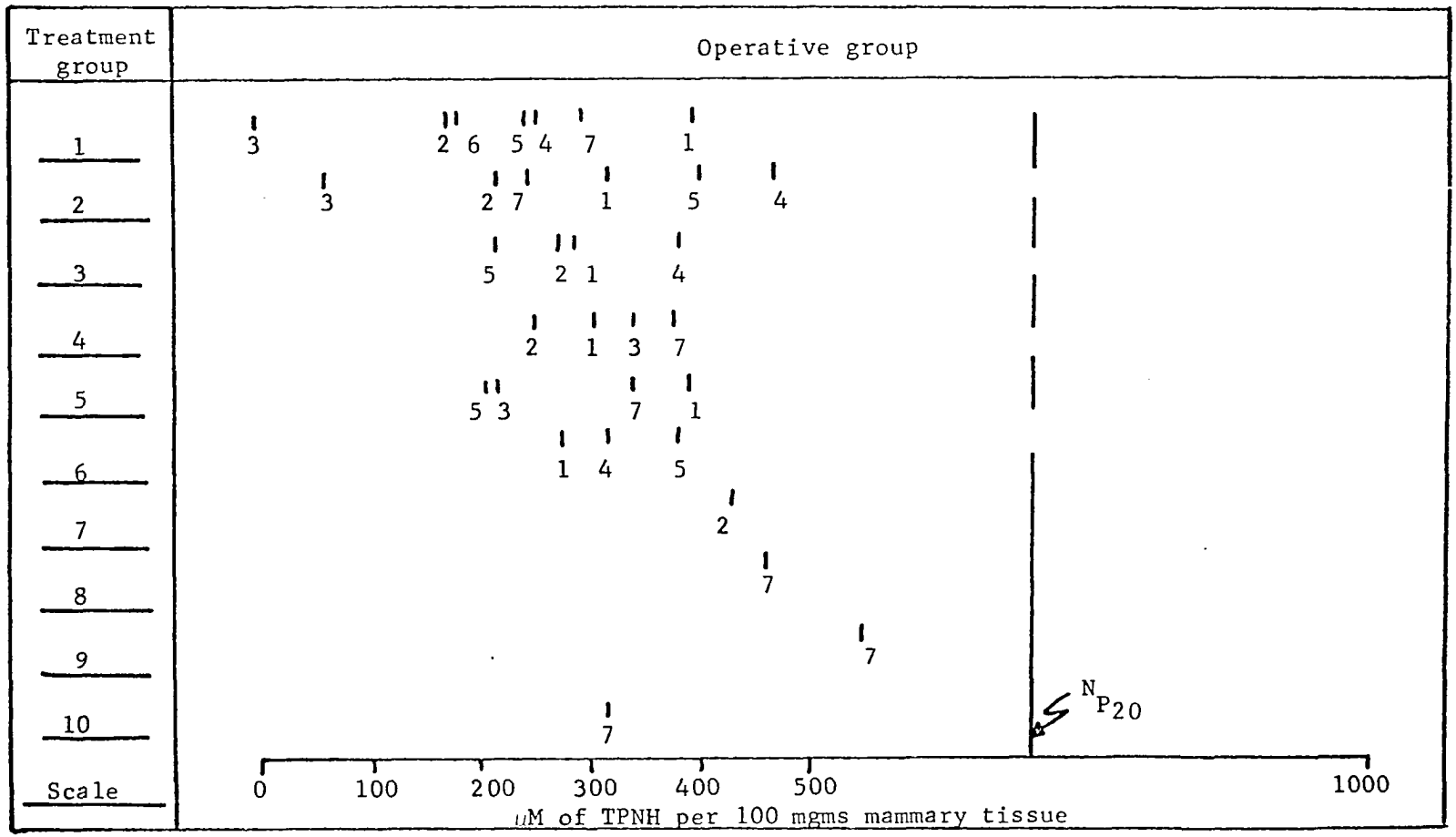

Figure 45. Graphic representation of TPNH results from Dunnett's test, stratified by treatment group

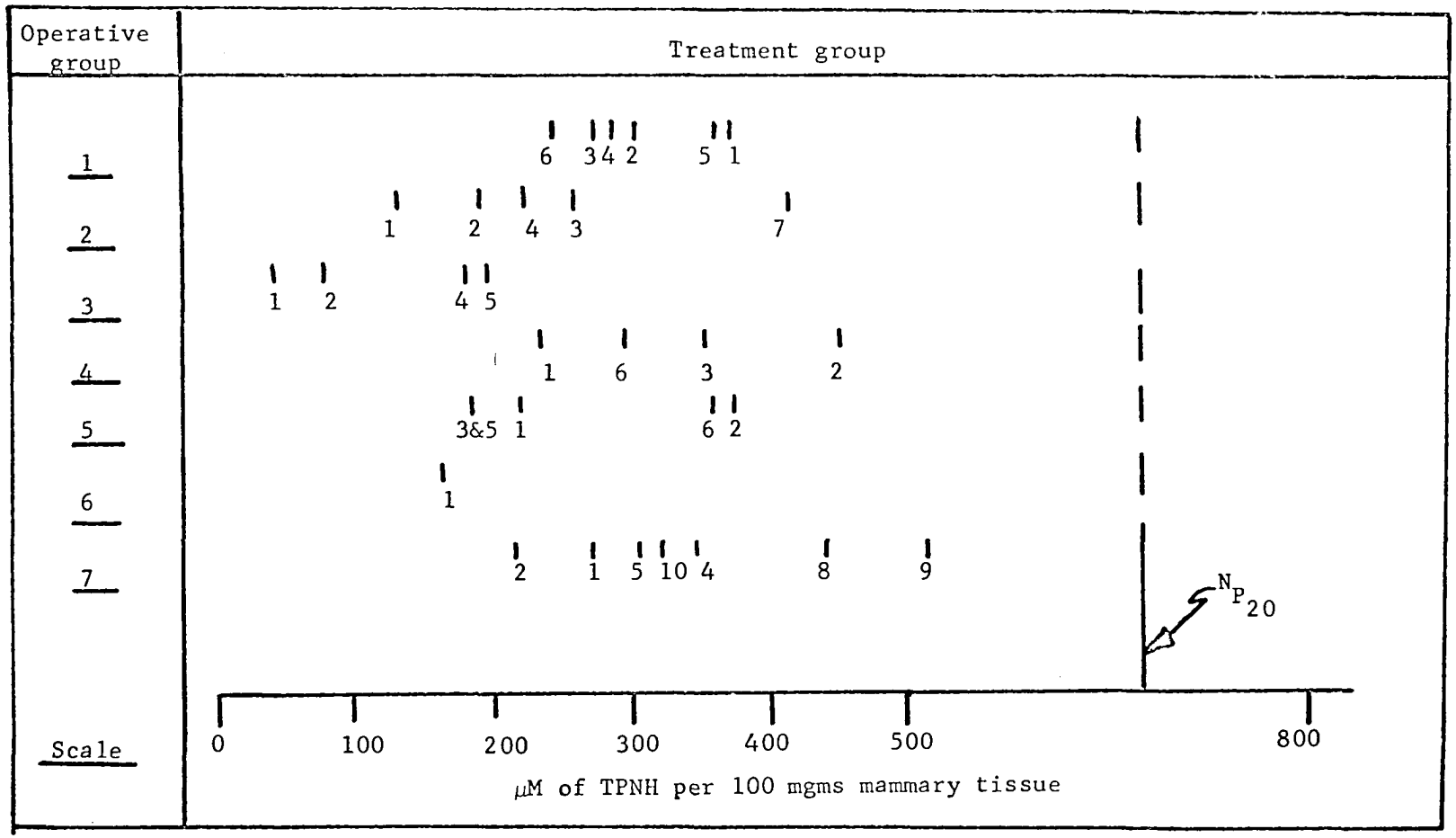

Figure 46. Graphic representation of TPNH results from Dunnett's test, stratified by operative group

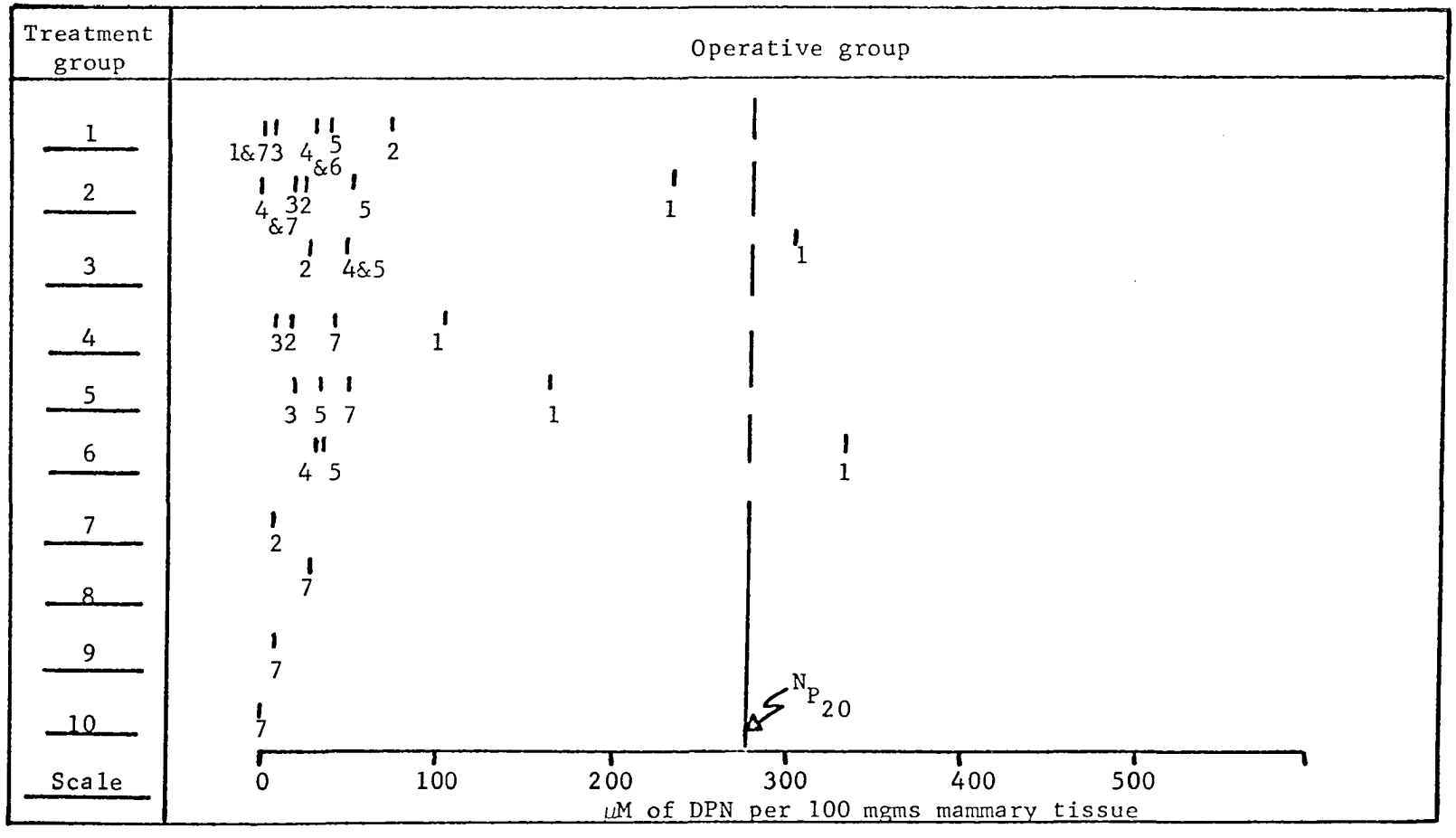

Figure 47. Graphic representation of DPN results from Dunnett's test, stratified by treatment group

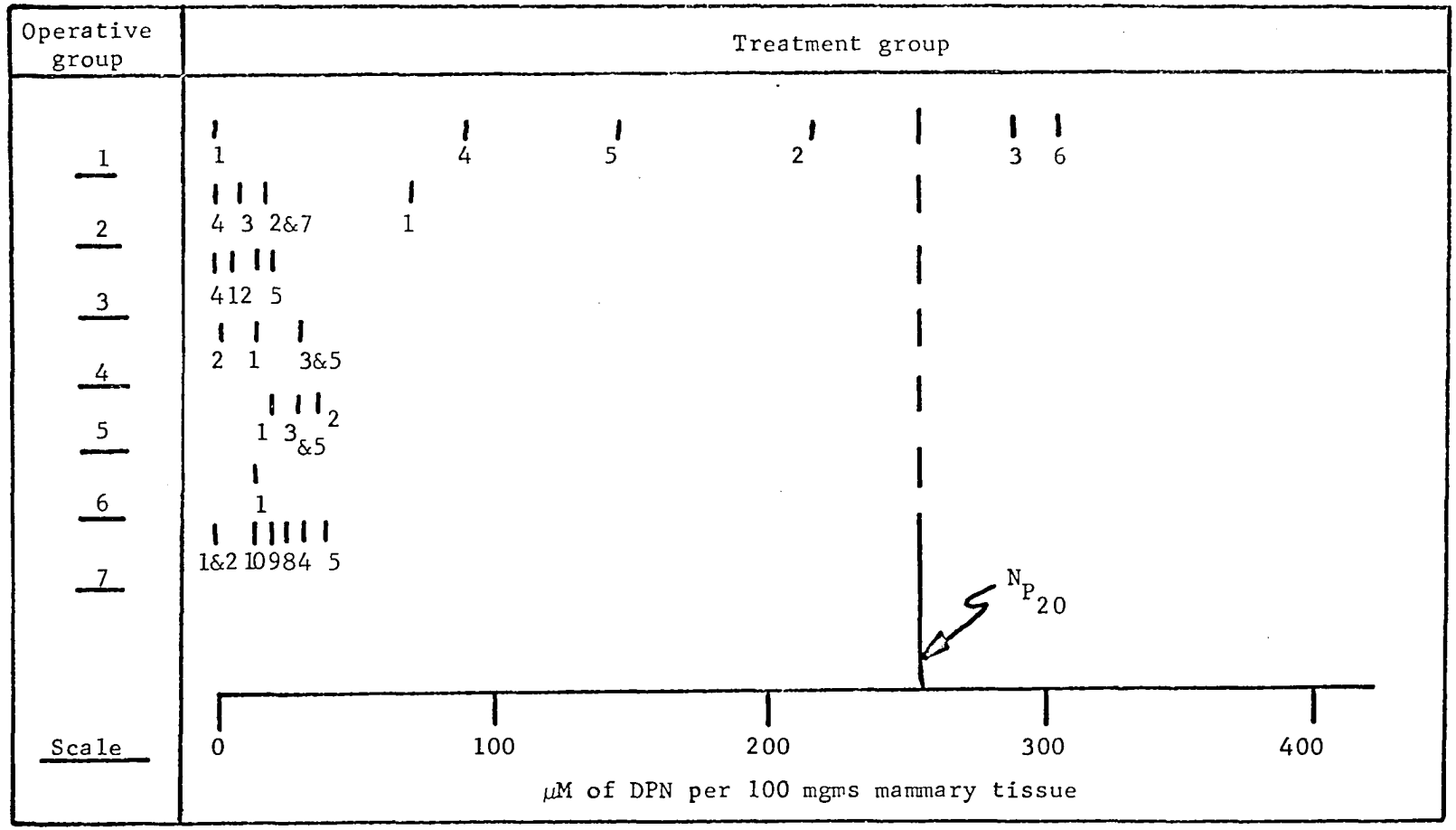

Figure 48. Graphic representation of DPN results from Dunnett's test, stratified by operative group

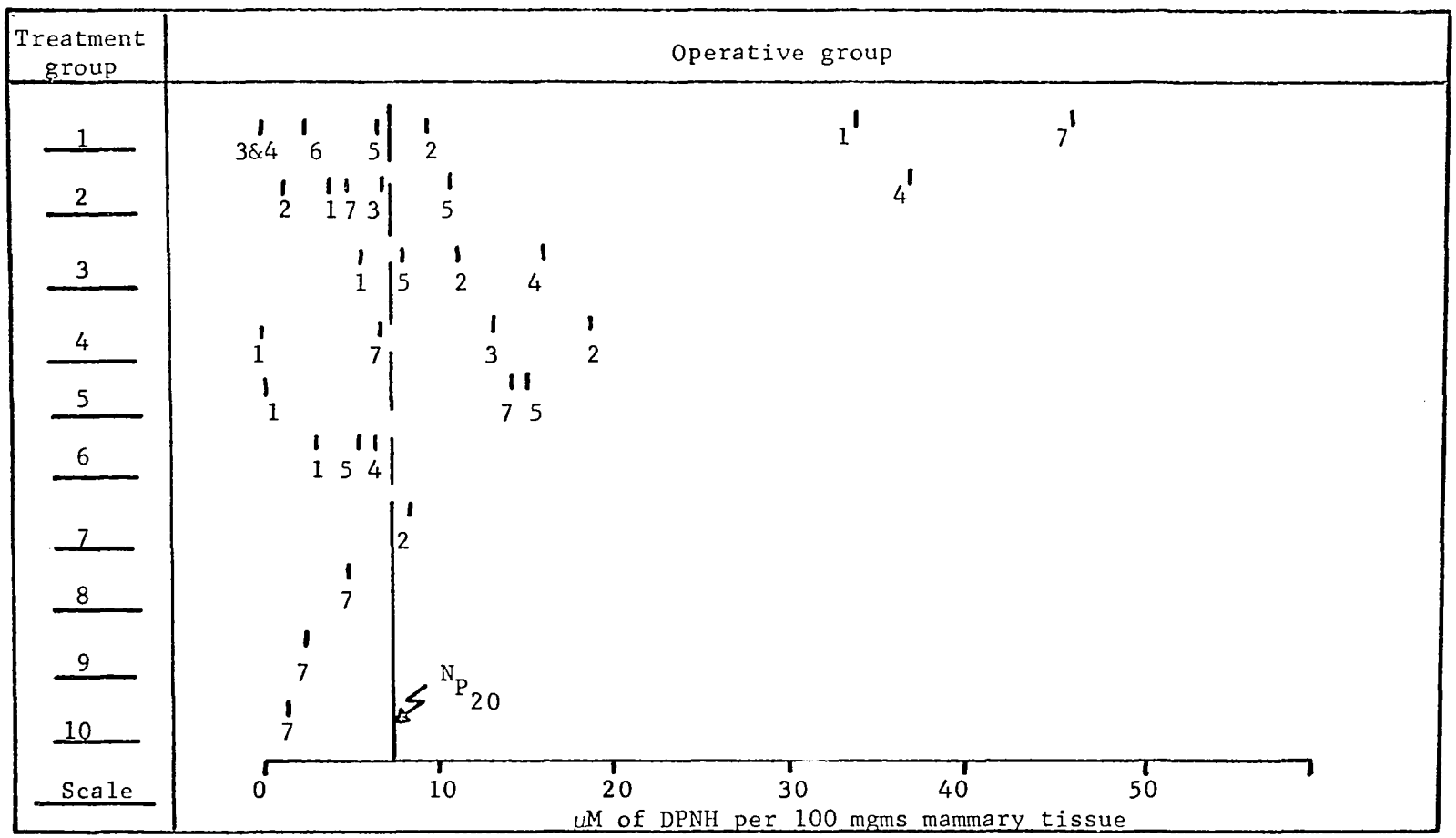

Figure 49. Graphic representation of DPNH results from Dunnett's test, stratified by treatment group

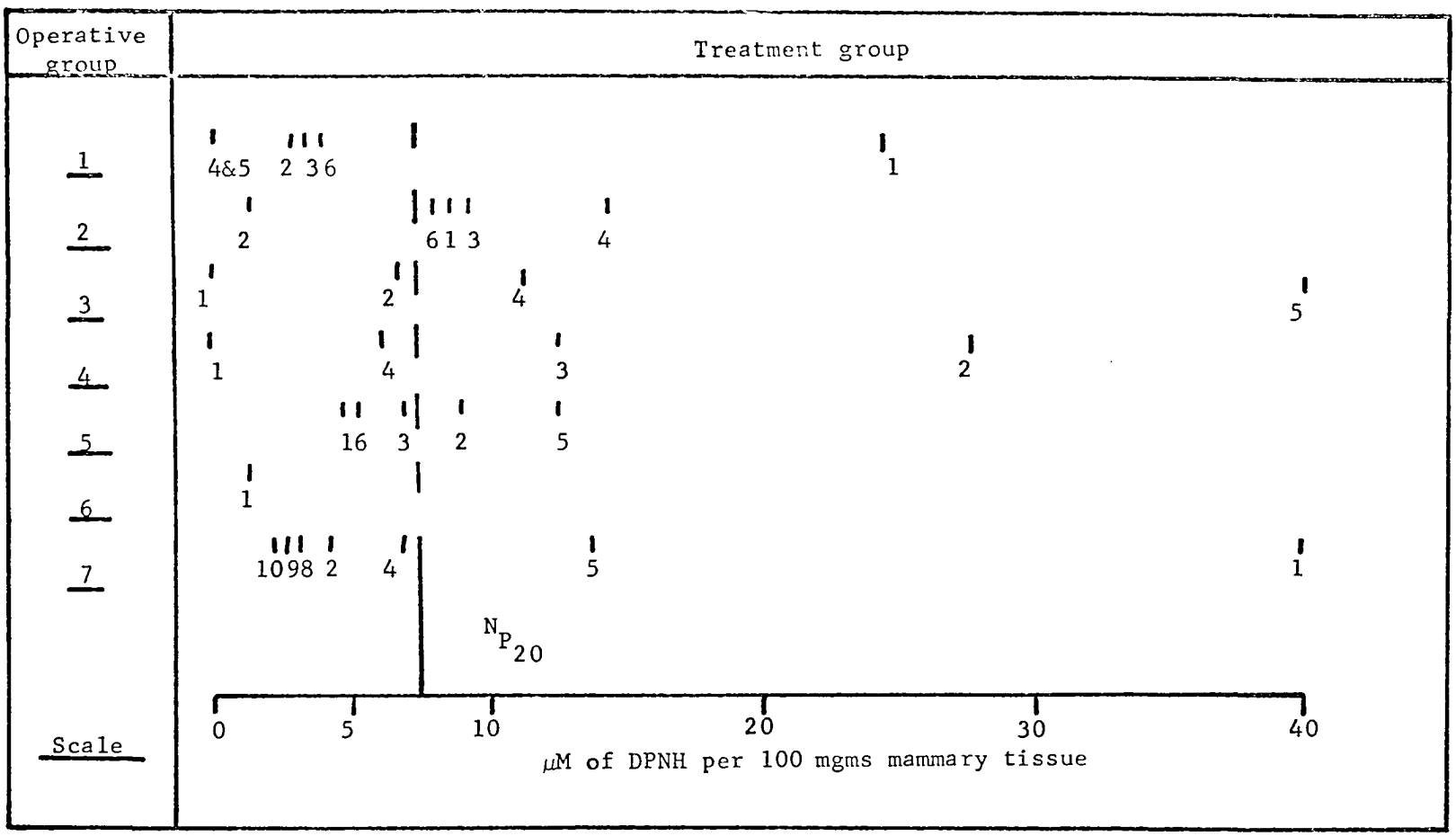

Figure 50. Graphic representation of DPNH results from Dunnett's test, stratified by operative group

trends when compared by the Dunnett test to the DPNH value at $N_{P_{20}}$.

Further investigation is necessary for a complete elucidation of the exact nature of these results. In order to attempt a tabular explanation, Chart 9 and Table 7 were compiled. Again using the above mentioned stratifications, these show the absolute effect differences resulting from the various treatments above (or below) that of N_C (i.e., $O_1 - T_1$), and from each other. From Chart 9 and Table 7, one is able to gain insight into the addition effect of particular hormones, given an operative state and a treatment condition. Because of the voluminous size of Chart 9 and Table 7, no explanation of individual effect differences or trends can be attempted.

Chart 9. Absolute treatment effects for treatments within operative groups

O_i	T_i	T_i mean value	N_c mean value	Corrected T_i ($T_i - N_c$)	Treatment differences $T_j - T_i$ ($j > i$)									
					T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
								1. RNA						
1	1	1.12	1.12	0.0	--	+2.75	+7.59	+5.99	+5.75	+8.18	--	--	--	--
	2	3.87		+2.75		--	+4.84	+3.24	+3.00	+5.43	--	--	--	--
	3	8.71		+7.59			--	-1.60	-1.84	+0.59	--	--	--	--
	4	6.11		+5.99				--	+0.24	-2.19	--	--	--	--
	5	6.87		+5.75					--	-2.43	--	--	--	--
	6	9.30		+8.18						--	--	--	--	--
2	1	1.20	1.12	+0.08	--	+3.58	+14.20	+9.11	--	--	+14.45	--	--	--
	2	4.78		+3.66		--	+10.62	+5.53	--	--	+10.87	--	--	--
	3	15.40		+14.28			--	-5.09	--	--	+0.25	--	--	--
	4	10.31		+9.19				--	--	--	+5.34	--	--	--
	7	15.65		+14.53							--	--	--	--
3	1	4.06	1.12	+2.94	--	+0.55	--	+7.59	+10.56	--	--	--	--	--
	2	4.61		+3.49		--	--	+7.04	+10.01	--	--	--	--	--
	4	11.65		+10.53				--	+2.97	--	--	--	--	--
	5	14.62		+13.50					--	--	--	--	--	--
4	1	1.65	1.12	+0.53	--	-0.29	-0.78	--	--	+1.14	--	--	--	--
	2	1.36		+0.24		--	-0.49	--	--	+1.43	--	--	--	--
	3	0.87		-0.25			--	--	--	+1.92	--	--	--	--
	6	2.79		+1.67						--	--	--	--	--
5	1	1.39	1.12	+0.27	--	-0.03	+0.19	--	-0.62	+1.00	--	--	--	--
	2	1.36		+0.24		--	+0.22	--	-0.59	+1.03	--	--	--	--
	3	1.58		+0.46			--	--	-0.81	+0.81	--	--	--	--
	5	0.77		-0.35					--	+1.62	--	--	--	--
	6	2.39		+1.27						--	--	--	--	--
6	1	1.29	1.12	+0.17	--	--	--	--	--	--	--	--	--	--
7	1	1.45	1.12	+0.33	--	-0.34	--	-0.40	-0.82	--	--	+2.24	+4.56	+1.31
	2	1.11		-0.01		--	--	-0.06	-0.48	--	--	+2.58	+4.90	+1.65
	4	1.05		-0.07				--	-0.42	--	--	+2.64	+4.96	+1.71
	5	0.63		-0.49					--	--	--	+3.06	+5.38	+2.13
	8	3.69		+2.57								--	+2.32	-0.93
	9	6.01		+4.89									--	-3.25
	10	2.76		+1.64										--
								2. DNA						
1	1	3.20	3.20	0.0	--	+2.80	+4.68	+2.55	+2.77	+3.46	--	--	--	--
	2	6.00		+2.80		--	+1.88	-0.25	-0.03	+0.66	--	--	--	--
	3	7.88		+4.68			--	-2.13	-1.91	+1.22	--	--	--	--
	4	5.75		+2.55				--	+0.22	+0.91	--	--	--	--
	5	5.97		+2.77					--	+0.69	--	--	--	--
	6	6.66		+3.46						--	--	--	--	--
2	1	3.12	3.20	-0.08	--	+4.12	+12.28	+7.19	--	--	+12.53	--	--	--
	2	7.24		+4.04		--	+8.16	+3.07	--	--	+8.41	--	--	--
	3	15.40		+12.20			--	-5.09	--	--	+0.25	--	--	--
	4	10.31		+7.11				--	--	--	+5.34	--	--	--
	7	15.65		+12.45							--	--	--	--
3	1	4.06	3.20	+0.86	--	+0.55	--	+7.59	+10.56	--	--	--	--	--
	2	4.61		+1.41		--	--	+7.04	+10.01	--	--	--	--	--
	4	11.65		+8.45				--	+2.97	--	--	--	--	--
	5	14.62		+11.42					--	--	--	--	--	--
4	1	1.65	3.20	-1.55	--	-0.29	+0.22	--	--	+1.20	--	--	--	--
	2	1.36		-1.84		--	+0.51	--	--	+1.49	--	--	--	--
	3	1.87		-1.33			--	--	--	+0.98	--	--	--	--
	6	2.85		-0.35						--	--	--	--	--
5	1	1.66	3.20	-1.54	--	+0.06	-0.01	--	-0.21	+0.76	--	--	--	--
	2	1.72		-1.48		--	-0.07	--	-0.27	+0.70	--	--	--	--
	3	1.65		-1.55			--	--	-0.20	+0.77	--	--	--	--
	5	1.45		-1.75					--	+0.97	--	--	--	--
	6	2.42		-0.78										
6	1	1.51	3.20	-1.69	--	--	--	--	--	--	--	--	--	--
7	1	2.05	3.20	-1.15	--	-0.15	--	-0.50	-0.38	--	--	+1.49	+3.98	+0.75
	2	1.90		-1.30		--	--	-0.35	-0.23	--	--	+1.64	+4.13	+0.90
	4	1.55		-1.65				--	+0.12	--	--	+1.99	+4.48	+1.25
	5	1.67		-1.53					--	--	--	+1.87	+4.36	+1.13

Chart 9. (Continued)

O_i	T_i	T_i mean value	N_c mean value	Corrected T_i ($T_i - N_c$)	Treatment differences $T_j - T_i$ ($j > i$)									
					T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
	8	3.54	+0.34									--	+2.49	-0.74
	9	6.03	+2.83										--	-3.23
	10	2.80	-0.40											--
								3. TPN						
1	1	525.8	525.8	0.0	--	-208.7	-197.7	-316.3	-126.8	-310.2	--	--	--	--
	2	317.1		-208.7		--	+11.0	-107.6	+81.9	-101.5	--	--	--	--
	3	328.1		-197.7			--	-118.6	+70.9	-112.5	--	--	--	--
	4	209.5		-316.3				--	+189.5	+6.1	--	--	--	--
	5	399.0		-126.8					--	-183.4	--	--	--	--
	6	215.6		-310.2						--	--	--	--	--
2	1	432.0	525.8	-93.8	--	-231.5	-200.2	-183.5	--	--	+195.3	--	--	--
	2	200.5		-325.3		--	+31.3	+48.0	--	--	+36.2	--	--	--
	3	231.8		-294.0			--	+16.7	--	--	+4.9	--	--	--
	4	248.5		-277.3				--	--	--	-11.8	--	--	--
	7	236.7		-289.1							--	--	--	--
3	1	176.8	525.8	-349.0	--	+16.0	--	+133.9	+77.7	--	--	--	--	--
	2	192.8		-333.0		--	--	+117.9	+61.7	--	--	--	--	--
	4	310.7		-215.1				--	-56.2	--	--	--	--	--
	5	254.5		-271.3					--	--	--	--	--	--
4	1	761.7	525.8	+234.9	--	-295.1	-211.6	--	--	-531.0	--	--	--	--
	2	465.6		-60.2		--	+36.9	--	--	-235.9	--	--	--	--
	3	502.5		-23.2			--	--	--	-272.8				
	6	229.7		-296.1						--	--	--	--	--
5	1	637.7	525.8	+111.9	--	+178.6	+67.9	--	-210.1	-321.2	--	--	--	--
	2	816.3		+290.5		--	-110.7	--	-388.7	-499.8	--	--	--	--
	3	705.6		+179.8			--	--	-278.0	-389.1	--	--	--	--
	5	427.6		-98.2					--	+111.1	--	--	--	--
	6	316.5		-209.3						--	--	--	--	--
6	1	629.5	525.8	+103.7	--	--	--	--	--	--	--	--	--	--
7	1	765.8	525.8	+210.0	--	+40.0	--	+240.1	+20.2	--	--	-176.1	-308.3	-583.3
	2	775.6		+250.0		--	--	+200.1	-19.8	--	--	-216.1	-348.2	-623.3
	4	975.9		+450.1				--	-219.8	--	--	-416.2	+548.3	-823.4
	5	756.0		+230.2					--	--	--	-196.3	-328.4	-503.5
	8	559.7		+33.9								--	-132.1	-407.2
	9	427.6		-98.2									--	-275.1
	10	152.5		-373.3										--
								4. TPNH						
1	1	373.3	373.3	0.0	--	-72.1	-97.7	-90.5	-6.2	-125.4	--	--	--	--
	2	301.2		-72.1		--	-25.6	-18.4	+65.9	-53.3	--	--	--	--
	3	275.6		-97.7			--	+7.2	+91.5	-27.7	--	--	--	--
	4	282.8		-90.5				--	+84.3	-34.9	--	--	--	--
	5	367.1		-6.2					--	-119.2	--	--	--	--
	6	247.9		-125.4						--	--	--	--	--
2	1	155.5	373.3	-217.8	--	+37.0	+104.1	+70.6	--	--	+261.1			
	2	192.5		-180.8		--	+67.1	+33.6	--	--	+224.1			
	3	259.6		-113.7			--	-33.5	--	--	+157.0			
	4	226.1		-147.2				--	--	--	+190.5			
	7	416.6		+43.3										
3	1	47.7	373.3	-325.6	--	+31.3	--	+135.6	+145.7	--	--	--	--	--
	2	79.0		-294.3		--	--	+104.3	+114.4	--	--	--	--	--
	4	183.3		-190.0				--	+10.1	--	--	--	--	--
	5	193.4		-179.9					--	--	--	--	--	--
4	1	236.7	373.3	-136.6	--	+214.9	+120.1	--	--	+59.6	--	--	--	--
	2	451.6		+78.3		--	-94.8	--	--	-155.3	--	--	--	--
	3	356.8		-16.5			--	--	--	-60.5	--	--	--	--
	6	296.3		-77.0						--	--	--	--	--
5	1	223.2	373.3	-150.1	--	+152.3	-32.7	--	-35.1	+140.8	--	--	--	--
	2	375.5		+2.2		--	-185.0	--	-187.4	-11.5	--	--	--	--
	3	190.5		-182.8			--	--	-2.4	+173.5	--	--	--	--
	5	188.1		-185.2					--	+175.9	--	--	--	--
	6	364.0		-9.3										

O_i	T_i	T_i mean value	N_c mean value	Corrected T_i ($T_i - N_c$)	Treatment differences $T_j - T_i$ ($j > i$)									
					T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
6	1	163.7	373.3	-209.6	--	--	--	--	--	--	--	--	--	--
7	1	275.7	373.3	-97.6	--	-56.1	--	+75.6	+34.6	--	--	+166.5	+241.7	+50.0
	2	219.6		-153.7		--	--	+131.7	+90.4	--	--	+222.6	+297.8	+106.1
	4	351.3		-22.0				--	-41.3	--	--	+90.9	+166.1	-25.6
	5	310.0		-63.3					--	--	--	+132.2	+207.4	+15.7
	8	442.2		+68.9								--	+75.2	+116.5
	9	517.4		+144.1									--	+191.7
	10	325.7		-47.6										--
					5. DPN									
1	1	0.0	0.0	0.0	--	+215.2	+290.2	+91.0	+145.8	+307.5	--	--	--	--
	2	215.2		+215.2		--	+75.0	-124.2	-69.4	+92.3	--	--	--	--
	3	290.2		+290.2			--	-199.2	-144.4	+17.3	--	--	--	--
	4	91.0		+91.0				--	+54.8	+216.5	--	--	--	--
	5	145.8		+145.8					--	+161.7	--	--	--	--
	6	307.5		+307.5										
2	1	70.7	0.0	+70.7	--	-55.1	-50.9	-70.7	--	--	-54.8	--	--	--
	2	15.6		+15.6		--	+4.2	-15.6	--	--	+0.3	--	--	--
	3	19.8		+19.8			--	-19.8	--	--	-3.7	--	--	--
	4	0.0		0.0				--	--	--	+15.9	--	--	--
	7	15.9		+15.9							--	--	--	--
3	1	6.5	0.0	+6.5	--	+8.4	--	-6.5	+15.1	--	--	--	--	--
	2	14.9		+14.9		--	--	-14.9	+6.7	--	--	--	--	--
	4	0.0		0.0				--	+21.6	--	--	--	--	--
	5	21.6		+21.6					--	--	--	--	--	--
4	1	15.7	0.0	+15.7	--	-15.7	+13.9	--	--	+13.8	--	--	--	--
	2	0.0		0.0		--	+29.6	--	--	+29.5	--	--	--	--
	3	29.6		+29.6			--	--	--	-0.1	--	--	--	--
	6	29.5		+29.5						--	--	--	--	--
5	1	19.5	0.0	+19.5	--	+16.6	+10.0	--	+12.2	+12.1	--	--	--	--
	2	36.1		+36.1		--	-6.6	--	-4.4	-4.5	--	--	--	--
	3	29.5		+29.5			--	--	+2.2	+2.1	--	--	--	--
	5	31.7		+31.7					--	-0.1	--	--	--	--
	6	31.6		+31.6						--	--	--	--	--
6	1	15.1	0.0	+15.1	--	--	--	--	--	--	--	--	--	--
7	1	0.0	0.0	0.0	--	0.0	--	+28.9	+39.9	--	--	+25.4	+19.6	+14.8
	2	0.0		0.0		--	--	+28.9	+39.9	--	--	+25.4	+19.6	+14.8
	4	28.9		+28.9				--	+11.0	--	--	-3.5	-9.3	-14.1
	5	39.9		+39.9					--	--	--	-14.5	-20.3	-10.6
	8	25.4		+25.4								--	-5.8	-10.8
	9	19.6		+19.6									--	-4.8
	10	14.8		+14.8										--
					6. DPNH									
1	1	24.6	24.6	0.0	--	-21.5	-21.2	-24.6	-24.6	-20.9	--	--	--	--
	2	3.1		-21.5		--	+0.3	-3.1	-3.1	+0.6	--	--	--	--
	3	3.4		-21.2			--	-3.4	-3.4	+0.3	--	--	--	--
	4	0.0		-24.6				--	0.0	+3.7	--	--	--	--
	5	0.0		-24.6					--	+3.7	--	--	--	--
	6	3.7		-20.9						--	--	--	--	--
2	1	8.4	24.6	-16.2	--	+6.9	+1.0	+6.0	--	--	-0.6	--	--	--
	2	1.5		-23.1		--	+7.9	+12.9	--	--	+6.3	--	--	--
	3	9.4		-15.2			--	+5.0	--	--	-1.6	--	--	--
	4	14.4		-10.2							-6.6	--	--	--
	7	7.8		-16.8							--	--	--	--
3	1	0.0	24.6	-24.6	--	+6.7	--	+11.3	+49.6	--	--	--	--	--
	2	6.7		-17.9		--	--	+4.6	+42.9	--	--	--	--	--
	4	11.3		-13.3				--	+38.3	--	--	--	--	--
	5	49.6		+25.0					--	--	--	--	--	--
4	1	0.0	24.6	-24.6	--	+27.6	+12.7	--	--	+6.7	--	--	--	--
	2	27.6		+3.0		--	-14.9	--	--	-20.9	--	--	--	--
	3	12.7		-11.9			--	--	--	-6.0	--	--	--	--
	6	6.7		-17.9						--	--	--	--	--

O_i	T_i	T_i mean value	N_c mean value	Corrected T_i ($T_i - N_c$)	Treatment differences $T_j - T_i$ ($j > i$)									
					T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
5	1	5.1	24.6	-19.6	--	+4.2	-2.6	--	+7.9	+0.5	--	--	--	--
	2	9.2		-15.4		--	-1.6	--	+3.7	-3.7	--	--	--	--
	3	7.6		-17.0			--	--	+5.3	-2.1	--	--	--	--
	5	12.9		-11.7					--	-7.4	--	--	--	--
	6	5.4		-19.1						--	--	--	--	--
6	1	1.7	24.6	-22.9	--	--	--	--	--	--	--	--	--	--
7	1	39.7	24.6	+15.1	--	-35.3	--	-32.7	-28.3	--	--	-36.5	-36.8	-37.1
	2	4.4		-20.2		--	--	+2.6	+7.0	--	--	-1.2	-1.5	-1.8
	4	7.0		-17.6				-	+4.4	--	--	-3.8	-4.1	-4.4
	5	11.4		-13.2					--	--	--	-8.2	-8.5	-8.8
	8	3.2		-21.4								--	-0.3	-0.6
	9	2.9		-21.7									--	-0.3
	10	2.6		-22.0										--

Table 7. Absolute operative effects for operative groups within treatments

T_i	O_i	O_i mean value	N_C mean value	Corrected O_i ($O_i - N_C$)	Operative differences $O_j - O_i$ ($j > i$)						
					O_1	O_2	O_3	O_4	O_5	O_6	O_7
						1. RNA					
1	1	1.12	1.12	0.0	--	+0.08	+2.94	+0.53	+0.27	+0.17	+0.33
	2	1.20		+0.08		--	+2.86	+0.45	+0.19	+0.09	+0.25
	3	4.06		+2.94			--	-2.41	-2.67	-2.77	-2.61
	4	1.65		+0.53				--	-0.26	-0.36	-0.20
	5	1.39		+0.27					--	-0.10	+0.06
	6	1.29		+0.17							+0.16
	7	1.45		+0.33							--
2	1	3.87	1.12	+2.75	--	+0.91	+0.74	-2.51	-2.51	--	-2.76
	2	4.78		+3.66		--	-0.17	-3.42	-3.42	--	-3.67
	3	4.61		+3.49			--	-3.25	-3.25	--	-3.50
	4	1.36		+0.24				--	0.0	--	-0.25
	5	1.36		-0.24							-0.25
	7	1.11		-0.01							--
3	1	8.71	1.12	+7.59	--	+7.59	--	-7.84	-6.13		
	2	15.40		+15.18		--	--	-15.43	-13.72		
	4	0.87		-0.25				--	+1.71		
	5	1.58		+1.46					--		
4	1	6.11	1.12	+4.99	--	+5.20	+6.54	--	--	--	-5.06
	2	10.31		+10.19		--	-1.34	--	--	--	-10.26
	3	11.65		+11.53			--	--	--	--	-11.60
	7	1.05		-0.07							--

Table 7. (Continued)

T_i	O_i	O_i mean value	N_C mean value	Corrected O_i ($O_i - N_C$)	Operative differences $O_j - O_i$ ($j > i$)						
					O_1	O_2	O_3	O_4	O_5	O_6	O_7
5	1	6.87	1.12	+6.75	--	--	+7.75	--	-7.10	--	-7.24
	3	14.62		+14.50			--	--	-14.85	--	-14.99
	5	0.77		-0.35					--	--	-0.15
	7	0.63		-0.49							--
6	1	9.30	1.12	+9.18	--	--	--	-7.51	-7.91		
	4	2.79		+1.67				--	-0.40		
	5	2.39		+1.27					--		
7	2	15.65	1.12	+15.53	--						
8	7	3.69	1.12	+2.57	--						
9	7	6.01	1.12	+4.89	--						
10	7	2.76	1.12	+1.64	--						
						2. DNA					
1	1	3.20	3.20	0	--	-0.08	+0.75	-1.23	-1.54	-1.69	-1.15
	2	3.12		-0.08		--	+0.83	-1.15	-1.46	-1.61	-1.07
	3	3.95		+0.75			--	-1.98	-2.29	-2.44	-1.90
	4	1.97		-1.23				--	-0.31	-0.46	+0.06
	5	1.66		-1.54					--	-0.15	+0.39
	6	1.51		-1.69						--	-0.54
	7	2.05		-1.15							--

Table 7. (Continued)

T_i	O_i	O_i mean value	N_C mean value	Corrected O_i ($O_i - N_C$)	Operative differences $O_j - O_i$ ($j > i$)						
					O_1	O_2	O_3	O_4	O_5	O_6	O_7
2	1	6.00	3.20	+2.80	--	+1.24	+1.33	-3.95	-4.28	--	-4.10
	2	7.24		+4.04		--	+0.09	-5.19	-5.52	--	-5.34
	3	7.33		+4.13			--	-5.28	-5.61	--	-5.43
	4	2.05		-1.15				--	-0.33	--	-0.15
	5	1.72		-1.48							+0.18
	7	1.90		-1.30							--
3	1	7.88	3.20	+4.68	--	+1.52	--	-6.01	-6.23		
	2	9.40		+6.20		--	--	-7.53	-7.75		
	4	1.87		-1.33				--	-0.22		
	5	1.65		-1.55					--		
4	1	5.75	3.20	+2.55	--	+1.02	+1.42	--	--	--	-4.70
	2	6.77		+3.57		--	+0.40	--	--	--	-5.72
	3	7.17		+3.97			--	--	--	--	-6.12
	7	1.05		-2.15							--
5	1	5.97	3.20	+2.77	--	--	+2.78	--	-4.52	--	-4.30
	3	8.75		+5.55			--	--	-7.30	--	-7.08
	5	1.45		-1.75					--	--	+0.22
	7	1.67		-1.53							--
6	1	6.66	3.20	+3.46	--	--	--	-3.81	-4.24		
	4	2.85		-0.35				--	-0.43		
	5	2.42		-0.78					--		
7	2	8.42	3.20	5.22	--						

Table 7. (Continued)

T_i	O_i	O_i mean value	N_C mean value	Corrected O_i ($O_i - N_C$)	Operative differences $O_j - O_i$ ($j > i$)						
					O_1	O_2	O_3	O_4	O_5	O_6	O_7
8	7	3.54	3.20	+0.34	--						
9	7	6.03	3.20	+2.83	--						
10	7	2.80	3.20	-0.40	--						
					3. TPN						
1	1	525.8	525.8	0	--	-93.8	-349.0	+235.9	+111.9	+103.7	+240.0
	2	432.0		-93.8		--	-255.2	+329.7	+205.7	+196.5	+333.8
	3	176.8		-349.0			--	+584.9	+460.9	+452.7	+589.0
	4	761.7		+235.9				--	-124.0	-132.7	+4.1
	5	637.7		+111.9					--	-8.2	+128.1
	6	629.5		+103.7						--	+136.3
	7	765.8		+240.0							--
2	1	317.1	525.8	-208.7	--	-116.6	-124.3	+148.5	+499.2	--	+458.5
	2	200.5		-325.3		--	-7.7	+265.1	+615.8	--	+575.1
	3	192.8		-333.0			--	+272.8	+623.5	--	+582.8
	4	465.6		-60.2				--	+350.7	--	+310.0
	5	816.3		+290.5			--	--	--	--	-40.7
	7	775.6		+249.8							--
3	1	328.1	525.8	-197.7	--	-96.3	--	+174.4	+377.5		
	2	231.8		-294.0		--	--	+270.7	+473.8		
	4	502.5		-23.3				--	+203.1		
	5	705.6		+179.8					--		

Table 7. (Continued)

T_i	O_i	O_i mean value	N_C mean value	Corrected O_i ($O_i - N_C$)	Operative differences $O_j - O_i$ ($j > i$)						
					O_1	O_2	O_3	O_4	O_5	O_6	O_7
4	1	209.5	525.8	-316.3	--	+39.0	+101.2	--	--	--	+766.4
	2	248.5		-277.3		--	+62.2	--	--	--	+727.4
	3	310.7		-215.1			--	--	--	--	+665.2
	7	975.9		+450.1							--
5	1	399.0	525.8	-126.8	--	--	-144.5	--	+28.6	--	+357.0
	3	254.5		-271.3			--	--	+173.1	--	+501.5
	5	427.6		-98.2					--	--	+328.4
	7	756.0		+230.2							--
6	1	215.6	525.8	-310.2	--	--	--	+14.1	+100.9		
	4	229.7		-296.1				--	+86.8		
	5	316.5		-209.3					--		
7	2	236.7	525.8	-289.1	--						
8	7	559.7	525.8	+33.9	--						
9	7	427.6	525.8	-98.2	--						
10	7	152.5	525.8	-373.3	--						
				4. TPNH							
1	1	373.3	373.3	0.0	--	-217.8	-325.6	-136.6	-150.1	-209.6	-97.6
	2	155.5		-217.8		--	-107.8	+81.2	+67.7	+8.2	+120.2
	3	47.7		-325.6			--	+189.0	+175.5	+116.0	+228.0

Table 7. (Continued)

T_i	O_i	O_i mean value	N_C mean value	Corrected O_i ($O_i - N_C$)	Operative differences $O_j - O_i$ ($j > i$)						
					O_1	O_2	O_3	O_4	O_5	O_6	O_7
	4	236.7		-136.6				--	-13.5	-73.0	+39.0
	5	223.2		-150.1					--	-59.0	+52.5
	6	163.7		-209.6						--	112.0
	7	275.7		-97.6							--
2	1	301.2	373.3	-97.6	--	-83.2	-196.7	+175.9	+99.8	--	-56.1
	2	192.5		-180.8		--	-113.5	+259.1	+183.0	--	+27.1
	3	79.0		-294.3			--	+372.6	+296.5	--	+140.6
	4	451.6		+78.3				--	-76.1	--	-232.0
	5	375.5		+2.2					--	--	-155.9
	7	219.6		-153.7							--
3	1	275.6	373.3	-97.7	--	-43.8	--	+226.9	+430.0		
	2	231.8		-141.5		--	--	+170.7	+475.8		
	4	502.5		+129.2				--	+203.1		
	5	705.6		+332.3					--		
4	1	282.8	373.3	-90.5	--	-56.7	-99.5	--	--	--	+68.5
	2	226.1		-147.2		--	-42.8	--	--	--	+125.2
	3	183.3		-190.0			--	--	--	--	+168.0
	7	351.3		-22.0							--
5	1	367.1	373.3	-6.2	--	--	-173.7	--	-179.0	--	-57.1
	3	193.4		-179.9			--	--	-5.3	--	+116.6
	5	188.1		-185.2					--	--	+121.9
	7	310.0		-63.3							--

Table 7. (Continued)

T_i	O_i	O_i mean value	N_C mean value	Corrected O_i ($O_i - N_C$)	Operative differences $O_j - O_i$ ($j > i$)						
					O_1	O_2	O_3	O_4	O_5	O_6	O_7
6	1	247.9	373.3	-125.4	--	--	--	+48.4	+116.1		
	4	296.3		-77.0				--	+67.7		
	5	364.0		-9.3					--		
7	2	416.6	373.3	+43.3	--						
8	7	442.2	373.3	+68.9	--						
9	7	517.4	373.3	+144.1	--						
10	7	325.7	373.3	-47.6	--						
						5. DPN					
1	1	0.0	0.0	0.0	--	+70.7	+6.5	+15.7	+19.5	+15.1	0.0
	2	70.7		70.7			-64.2	-55.0	-51.2	-55.6	-70.7
	3	6.5		6.5			--	+9.2	+13.0	+8.6	-6.5
	4	15.7		15.7				--	+3.8	-0.6	-15.7
	5	19.5		19.5					--	-4.4	-19.5
	6	15.1		15.1						--	-15.1
	7	0.0		0.0							--
2	1	215.2	0.0	215.2	--	-199.6	-200.3	-215.2	-179.1	--	-215.2
	2	15.6		15.6		--	-0.7	-15.6	+20.5	--	-15.6
	3	14.9		14.9			--	-14.9	+21.2	--	-14.9
	4	0.0		0.0				--	+36.1	--	0.0
	5	36.1		36.1							-36.1
	7	0.0		0.0							--

Table 7. (Continued)

T_i	O_i	O_i mean value	N_C mean value	Corrected O_i $(O_i - N_C)$	Operative differences $O_j - O_i$ $(j > i)$						
					O_1	O_2	O_3	O_4	O_5	O_6	O_7
3	1	290.2	0.0	290.2	--	-270.4	--	-260.6	-260.7		
	2	19.8		19.8		--	--	+9.8	+9.7		
	4	29.6		29.6				--	-0.1		
	5	29.5		29.5					--		
4	1	91.0	0.0	91.0	--	-91.0	-91.0	--	--	--	-62.1
	2	0.0		0.0		--	0.0	--	--	--	+28.9
	3	0.0		0.0			--	--	--	--	+28.9
	7	28.9		28.9							--
5	1	145.8	0.0	145.8	--	--	-124.2	--	-114.1	--	-105.9
	3	21.6		21.6			--	--	+10.1	--	+18.3
	5	31.7		31.7					--	--	+8.2
	7	39.9		39.9							--
6	1	307.5	0.0	307.5	--	--	--	-278.0	-275.9		
	4	29.5		29.5				--	+2.1		
	5	31.6		31.6					--		
7	2	15.9	0.0	15.9	--						
8	7	25.4	0.0	25.4	--						
9	7	19.6	0.0	19.6	--						
10	7	14.8	0.0	14.8	--						

Table 7. (Continued)

T_i	O_i	O_i mean value	N_C mean value	Corrected O_i ($O_i - N_C$)	Operative differences $O_j - O_i$ ($j > i$)						
					O_1	O_2	O_3	O_4	O_5	O_6	O_7
					6. DPNH						
1	1	24.6	24.6	0.0	--	-16.2	-24.6	-24.6	-19.5	-22.9	+15.1
	2	8.4		-16.2		--	-8.4	-8.4	-3.3	-6.7	+31.3
	3	0.0		-24.6			--	0.0	+5.1	+1.7	+39.7
	4	0.0		-24.6				--	+5.1	+1.7	+39.7
	5	5.1		-19.5					--	-5.4	+34.6
	6	1.7		-22.9						--	+38.0
	7	39.7		+15.1							--
2	1	3.1	24.6	-21.5	--	-1.6	+3.6	+24.5	+6.1	--	+1.3
	2	1.5		-23.1		--	+5.2	+26.1	+7.7	--	+2.9
	3	6.7		-17.9			--	+20.9	+2.5	--	-2.3
	4	27.6		+3.0				--	-18.4	--	-23.2
	5	9.2		-15.4					--	--	-4.8
	7	4.4		-20.2							--
3	1	3.4	24.6	-21.2	--	+6.0	--	+9.3	+4.2		
	2	9.4		-15.2		--	--	+3.3	-1.8		
	4	12.7		-11.9				--	-5.1		
	5	7.6		-17.0					--		
4	1	0.0	24.6	-24.6	--	+14.4	+11.3	--	--	--	+28.9
	2	14.4		-10.2		--	-3.1	--	--	--	+14.5
	3	11.3		-13.3			--	--	--	--	+17.6
	7	28.9		+4.3							--
5	1	0.0	24.6	-24.6	--	--	+49.6	--	+12.9	--	+11.4
	3	49.6		+25.0			--	--	-36.7	--	-38.2
	5	12.9		-11.7					--	--	-1.5
	7	11.4		-13.2							--

Table 7. (Continued)

T_i	O_i	O_i mean value	N_C mean value	Corrected O_i $(O_i - N_C)$	Operative differences $O_j - O_i$ $(j > i)$						
					O_1	O_2	O_3	O_4	O_5	O_6	O_7
6	1	3.7	24.6	-20.9	--	--	--	+3.0	+2.0		
	4	6.7		-17.9				--	-1.0		
	5	5.7		-18.9					--		
7	2	7.8	24.6	-16.8	--						
8	7	3.2	24.6	-21.4	--						
9	7	2.9	24.6	-21.7	--						
10	7	2.6	24.6	-22.0	--						

VI. DISCUSSION

The data shown in a previous section are in agreement with those found in the literature, for comparable experimentation. Since the purpose of this dissertation is to attempt assimulation of the biochemical-endocrinological interrelationships, a discussion of natural and artificial mammogenesis, as was observed from the results of the involved substances, is necessary.

From the present investigations, it appears that the biochemistry of the gland is in a dynamic, variable state owing to conception, to the hormonal changes during gestation and due to parturition. In addition, it appears that the most critical indications of mammary gland physiology are the quantitative measurements of DNA, RNA/DNA, DPN/DONH and TPNH/TPN. These indices express the extent of new cell formation in the gland, of proteidogenesis for the gland, of the energy capabilities of the gland via the electron transport system, and of the relative rate of lipogenesis in the gland, respectively. Thus, to simplify this discussion only these indices of glandular function will be considered.

A. Natural Mammogenesis

The early work of Roberts (161) suggested that mammary gland development occurred in two stages. The first stage, consisting primarily of hyperplasia and hypertrophy, occurred up to the 13th day of pregnancy, and the second stage, consisting of secretory modification and enlargement of the alveoli, continued through the remainder of pregnancy and into lactation. This process was considered to be reversed with the

start of involution.

In this discussion P_i and L_i will be defined as the ith day of pregnancy and lactation, respectively.

1. DNA

The criterion of DNA measurement as a quantitative indication of cellular development in the gland has shown the work of Roberts (161) is inaccurate. Using the DNA criterion, the results herein indicate that glandular growth occurs both during pregnancy and lactation. This observation is in agreement with more recent investigations (76, 77, 141). The present findings indicate that the most rapid increase in total cells formed, during pregnancy, occurs between P_{10} and P_{15} (P_{10} - P_{15}). In this time interval approximately 50 percent of all new cells formed during pregnancy and 30 percent of all cells formed during pregnancy and lactation are produced, as shown in Table 8. The percentage values shown in Table 8 were obtained by subtracting the lowest DNA reading, i.e., that at P_0, from each of the remaining DNA values and then calculating all values as a percentage of the highest correct value, i.e., L_{20}.

One can also observe from Table 8, that by P_{15}, approximately 45 percent of all new cells produced during these two physiological periods are formed. Prior to P_{10}, only 14 percent of the new cells are formed, and between the interval P_{15} - P_{20} there is only a 16 percent increase in new cells, giving a total of nearly 60 percent of all cells produced, both in pregnancy and lactation, are present by P_{20}.

A comparable period of rapid cellular formation during lactation

occurs between the interval L_1 - L_5. Within this time interval 70 percent of all new cells produced during lactation are formed, and approximately 32 percent of all new cells formed during both physiological periods are present.

If we consider the total production of new cells during both periods, it is observed that by L_5, there are present 92 percent of all cells produced. After L_5 there appears to be a damping out of cellular production through the remainder of lactation, with the exception of the interval L_{15} - L_{20}. This "leveling-off" is probably due to the attainment of maximal mammary gland size under the "existing" normal nursing conditions. The final "spurt", during the interval L_{15} - L_{20}, is probably related to continued nursing past L_{15}, as suggested by Tucker and Reece (180) as was allowed in this investigation.

2. RNA-DNA ratio

Maximal proteidogenesis, as measured by the ratio of RNA per DNA, occurs during late pregnancy and continues throughout lactation, as shown in Table 17. The most rapid increase in protein synthesizing abilities within pregnancy occurs between P_{15} - P_{20}. During this interval there is an 11 percent increase. Prior to P_{15}, the greatest increase is approximately 6 percent that occurs between P_0 - P_5. This early increase is probably related to a general overall increase in proteidogenesis due to the protein requirements of conception and initial pregnancy. This view is suggested by the reduction in proteidogenesis between P_5 - P_{10}.

Table 8. Percentage of maximum value during pregnancy and lactation (data corrected from Chart 1)

Considered period	Percentage of maximum corrected value			
	DNA	RNA/DNA	DPN/DPNH	TPNH/TPN
N_{P_0}	0	0	0	0
N_{P_5}	2.80	5.69	8.55	5.71
$N_{P_{10}}$	14.23	3.25	87.81	42.95
$N_{P_{15}}$	43.47	6.50	61.07	49.79
$N_{P_{20}}$	59.74	17.48	37.60	4.19
N_{L_1}	67.24	31.30	100	52.67
N_{L_5}	92.40	48.37	33.01	62.30
$N_{L_{10}}$	93.93	76.83	94.08	80.22
$N_{L_{15}}$	91.89	90.24	23.27	100
$N_{L_{20}}$	100	100	9.35	51.45

Protein synthesis during lactation continues at a high rate, having a sigmoidal functional relation through time. This relation suggests proteidogenesis is required to maintain adequate milk protein to properly nourish the young. The reduction in rate of protein synthesis, after L_{15}, is probably related to other food sources for the litter. However, there was continued nursing beyond L_{15} which may account for the continued increase of both RNA and DNA; the relative increase of RNA twice as high as that of DNA.

3. DPN-DPNH ratio

Glock and McLean (67) and McLean (118,119,120) suggested that the ratio of DPNH to DPN was an indication of energy requirements of the cell, obtained through oxidative phosphylation. Although values were obtained similar to those of these investigators, the reciprocal is used here to imply their meaning since it is felt that the concentration of DPN is a direct result of this oxidation having occurred. In this way, it is felt, any increase in this ratio value, given variations in both DPN and DPNH, is an indication of periods of high oxidative phosphorylation. The data presented here suggest that the mammary gland needs increased energy sources during mid and late pregnancy, P_5 - P_{10} and P_{20} - L_1, and during lactation, L_5 - L_{10}. These are, as discussed above associated with periods prior to a most rapid increase in either the number of cell in the gland, during early pregnancy, and/or to maximal proteidogenesis, during late pregnancy and lactation.

4. TPNH-TPN ratio

Since this ratio as suggested by Glock and McLean (67) is indicative of potential synthesizing capacity of the gland, it is suggested by the present data that lipogenesis occurs both in pregnancy and in lactation. The rate of increase in regard to this ratio value is maximal during pregnancy between P_5 - P_{10}, with maximal lipogenesis, as measured by the ratio, occurring at P_{15}. In lactation, we find that the maximal rate of increase is between P_{20} - L_1. From L_1 - L_{15}, lipogenesis is increasing at a constant rate, with a decrease after L_{15}. This is believed to be related to the start of weaning.

5. Biochemical relationships of periods

The total generalized picture, as suggested by the above discussion, indicates that mammary glands of pregnancy and lactation are, seemingly biochemically, different. During pregnancy the gland is increasing in numbers of cells, has a low rate of proteidogenesis, has a high rate of lipogenesis and has a relatively high energy requirement. This physiological period of the gland is equivalent to adipose tissue undergoing fat storage from fatty acids (44, 69, 70, 203). One can envisage that during early and mid-pregnancy the primarily biochemical function of the mammary gland is lipogenesis related to the formation of structural adipose tissue. Late pregnancy is concerned with a reduction in lipogenesis and an increase in protein synthesis. This period is believed to be related to maximal tubular development in the gland.

During lactation, the gland continues to increase in total numbers of cells, with a high rate of both proteidogenesis and lipogenesis and maintains a high energy requirement. Lactation suggests a gland with a major biochemical function related to the formation of proteins and fats for nutritional purposes in milk (28, 45).

B. Artificial Mammogenesis

The present data for artificial mammogenesis will be similarly discussed in regards to the indices of gland function. These indices are found as a percentage of their respective value for $N_{P_{20}}$ in Table 9. In this way a comparison to the natural process using the criterion established in the literature can be made.

1. Normal animals (O_1)

a. T_1 This subgroup consisting of intact, normal animals, receiving no hormonal treatment. The observed percentage values of 41%, 44%, 0.0 and 43%, gives us the lower bases unit of these indices prior to proliferation due to gestation.

b. T_2 When this subgroup, receiving E and P, is compared to the above data obtained from pregnant and lactating animals, we observe that the gland has increased in the total number of cells from N_{P_0}, with relatively high protein synthesizing capacity, having a relatively high energy requirement, and a low rate of lipogenesis when compared to $N_{P_{20}}$. These observations are suggested from the individual percentage values of 76% (DNA), 82% (RNA/DNA), 188% (DPN/DPNH) and 57% (TPNH/TPN), as shown in Table 9.

c. T_3 This subgroup received E, P and T. The percentage values of 100%, 141%, 232% and 50%, suggest a gland that has increased cell numbers equal to $N_{P_{20}}$, with a relatively high capacity for proteidogenesis, a very high energy requirement, and a very low rate of lipogenesis.

d. T_4 The percentage values of 73%, 136%, infinity, and 81%, suggest a gland increased in size from N_{P_0}, with relative high protein synthesizing abilities, an extremely high energy requirement, and a relatively high rate of lipogenesis. This subgroup received, E, P, D and H.

e. T_5 This subgroup received E, P, D, H and T. The respective percentage values of 76%, 147%, infinity and 55% suggest an increased total number of cells, relatively high protein synthesizing capabilities,

Table 9. Indices of gland function for various operative-treatment groups

Considered groups $O_i - T_j$	Indices of gland function compared to $N_{P_{20}}$			
	DNA	RNA/DNA	DPN/DPNH	TPNH/TPN
$O_1 - T_1$	0.41	0.44	0.0	0.43
$- T^1_2$	0.76	0.82	1.88	0.57
$- T^2_3$	1.00	1.41	2.32	0.50
$- T^3_4$	0.73	1.36	infinity	0.81
$- T^4_5$	0.76	1.47	infinity	0.55
$- T^5_6$	0.84	1.79	2.25	0.69
$O_2 - T_1$	0.39	0.49	0.23	0.22
$- T^1_2$	0.92	0.85	0.28	0.57
$- T^2_3$	1.19	0.82	0.06	0.67
$- T^3_4$	0.86	1.95	0.0	0.55
$- T^4_7$	1.07	2.38	0.06	1.05
$O_3 - T_1$	0.50	1.31	infinity	0.16
$- T^1_2$	0.93	0.81	0.06	0.24
$- T^2_4$	0.91	2.08	0.0	0.35
$- T^4_5$	1.11	2.09	0.01	0.46
$O_4 - T_1$	0.24	1.08	infinity	0.19
$- T^1_2$	0.26	0.85	0.0	0.58
$- T^2_3$	0.24	0.60	0.06	0.43
$- T^3_6$	0.36	1.24	0.12	0.77
$O_5 - T_1$	0.21	1.08	0.10	0.22
$- T^1_2$	0.22	1.01	0.11	0.29
$- T^2_3$	0.21	1.23	0.11	0.16
$- T^3_5$	0.18	0.68	0.07	0.26
$- T^5_6$	0.31	1.27	0.16	0.69
$O_6 - T_1$	0.19	1.09	0.24	0.16
$O_7 - T_1$	0.26	0.91	0.0	0.22
$- T^1_2$	0.24	0.74	0.0	0.16
$- T^2_4$	0.20	0.87	0.11	0.22
$- T^4_5$	0.21	0.49	0.09	0.24
$- T^5_8$	0.44	1.33	0.22	0.47
$- T^8_9$	0.76	1.28	0.18	0.72
$- T_{10}$	0.35	1.27	0.15	1.28

extremely large energy requirements and a low rate of lipogenesis.

f. T_6 This subgroup received E, P, S, M and T. The percentage values of 84%, 179%, 225% and 69% suggest a gland similar to the normal gland during mid-pregnancy, such a gland is characterized by an increase in cell numbers, high energy requirement, having a very high protein producing capacity, and a rather low rate of lipogenesis.

2. Ovarectomized animals (O_2)

a. T_1 Ovarectomized animals receiving no hormonal treatment appeared to be similar physiologically to the normal, untreated control N_{P_0}, except they had higher energy requirements and a much lower rate (50 percent) of lipogenesis.

b. T_2 This subgroup was similar to $O_1 - T_2$, but had a much lower energy requirement (approximately eight times lower).

c. T_3 This subgroup suggested a gland with very high proliferation, very good proteidogenetic capabilities, very low energy needs, but a high rate of lipogenesis.

d. T_4 This subgroup had a relatively high rate of proliferation and high protein synthesizing capabilities, but an absence of energy from the electron transport system (possibly another source of energy is available), and a low rate of lipogenesis.

e. T_7 This subgroup, receiving E, P, S and M, suggested a gland with increased total cell numbers, the highest capacity to synthesize proteins, extremely low energy requirements and potential equivalent lipogenetic abilities potentially equivalent to those of $N_{P_{20}}$.

3. Adrenalectomized-ovarectomized animals (O_3)

a. T_1 This subgroup had very little proliferation, relatively high proteidogenetic capacities, extremely high energy requirements, but an extremely low rate of lipogenesis.

b. T_2 This subgroup had a high rate of cellular formation, relatively high protein synthesizing capacities, little or no energy requirements, and a low lipogenetic rate.

c. T_4 This subgroup was similar to O_3 - T_2, but had an extremely high rate of proteidogenesis.

d. T_5 This subgroup was relatively identical to O_3 - T_4, except having a higher level of proliferation.

4. Hypophysectomized-ovarectomized animals (O_4)

a. T_1 The data from this subgroup suggested that there was degeneration of the cells from N_{P_0}, but increased proteidogenetic abilities, an extremely high energy requirement, and a low lipogenetic rate.

b. T_2 This subgroup indicated a degeneration of cells resulting in decrease in number little or no energy requirement from the electron transport system, a low rate of lipogenesis, but a relatively high rate of protein synthesis.

c. T_3 This subgroup was similar to O_4 - T_2, but a lower rate of proteidogenesis.

d. T_6 This subgroup, likewise, suggested cellular deterioration or, destruction, with a relatively high protein and lipid synthesizing

capacity for the existing cells, but having low energy requirements.

5. Hypophysectomized animals (O_5)

Similar to O_4, all animals in this group indicated a decrease in the total number of cells from N_{P_0}. This group, in general, suggested low energy requirements, a low rate of lipogenesis, but high proteidogenetic capabilities (with the exception of O_5 - T_5, which had low rate of proteidogenesis).

6. Adrenalectomized-hypophysectomized animals (O_6)

This group of animals, receiving no hormonal injections, was similar to O_5, (cellular decrease, a high rate of proteidogenesis, low energy requirements, and a low rate of lipogenesis).

7. Adrenalectomized-hypophysectomized-ovarectomized animals (O_7)

This group, in general, had low energy requirements and a decrease in total cell numbers. The other two indices were variable with treatment.

a. T_1 This subgroup had moderate protein synthesizing capabilities and a low rate of lipogenesis.

b. T_2 This subgroup was identical to O_7 - T_1 in regard to rates of proteidogenesis and lipogenesis.

c. T_4 This subgroup was also similar in regards to the other two indices to O_7 - T_1.

d. T_5 This subgroup had a low potential for synthesizing protein, being equivalent to N_{P_0}, and had a low lipogenetic rate.

e. T_8 This subgroup had a relatively high rate for proteidogenesis and a relatively low rate for lipogenesis. This subgroup received E, P, D, H, S, and T.

f. T_9 This subgroup received E, P, D, H, M, and T had a relatively high rate of both proteidogenesis and lipogenesis. In addition, this subgroup had an increase in total cell numbers over N_{P_0}, but less than $N_{P_{20}}$.

g. T_{10} This subgroup had a relatively high rate of protein synthesis and a very high rate of lipogenesis. This subgroup receive as hormonal treatment E, P, D, H, S, M, and T.

8. Biochemical relationships of operative-treatment groups

The data presented herein indicates that while visually, as shown in the histological preparations, there appears equivalence between certain of the operative-treatment groups and $N_{P_{20}}$, and while one or more of the indices of gland function may, likewise, be similar to $N_{P_{20}}$, there is no one experimental group equivalent to $N_{P_{20}}$ for all indices. These observations are suggested by Table 9. Thus if we use $N_{P_{20}}$ as the a priori criterion of maximal glandular growth, there is no hormonal preparation studied in this limited series of experiments that will produce a gland functioning as a normal one in very late pregnancy for all indices. It would appear that one or more factors are missing from the present experimentation to make normal glandular growth impossible. The work of Willmer (202), reflects this view in his work on the bio-

chemical effects resulting from adrenalectomy and replacement hormonal therapy. It seems that a more thorough investigation of the synergistic actions of endocrine glands, their hormones and the important biochemical factors is necessary before a full understanding of the changes occurring during pregnancy and/or lactation can be obtained.

VII. SUMMARY

The present experimentation was concerned with the biochemical-endocrinological interrelationships of the mammary gland of the rat. To accomplish this purpose, four indications of gland function were advanced as a criterion. These indices were the quantitative measurements of DNA, RNA/DNA, DPN/DPNH, and TPNH/TPN as they express the extent of new cell formation in the gland, of proteidogenesis for the gland, of the energy capabilities of the gland via the election transport system, and of the relative rate of lipogenesis in the gland, respectively.

The results given herein suggest the following:

1. That during early and mid-pregnancy the mammary gland is acting similar to adipose tissue primarily for the storage of fat.

2. That during late pregnancy the gland is actively undergoing new cell formation primarily related to the formation of tubular structures, but to some alveolar proliferation.

3. That parturition stimulates great activity in the gland primarily related to the formation of proteins and fats for nutritional purposes, but also concerned with continued alveolar proliferation.

4. That early and mid-lactation continue to reflect the glandular changes stimulated by parturition.

5. That late lactation, as believed related to the start of weaning, reflects a decrease in the rate of lipogenesis and a "leveling-off" in the rates of proteidogenesis, as an attempt to begin involution.

6. That the periods of pregnancy and lactation are physiologically and biochemically different, due to requirements of the gland during

these periods.

7. That when normal and surgically ablated, non-pregnant rats with or without varying hormonal therapy are compared to normal, twenty day pregnant animals, $N_{P_{20}}$, no operative-treatment group is similar for all measured indices of glandular function.

The present experimentation suggests that more involved experimentation is necessary to understand the complete interactions of hormones on the physiology and biochemistry of the gland during various stages of growth and function.

VIII. LITERATURE CITED

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X. APPENDIX

A. Chemical Methodology

The following preparatory, analytical, and histological methods were used in this experimentation.

1. DNA and RNA determinations (150)

   a. Mammary gland tissue was frozen for not less than 4 days to remove excess water.

   b. After 4 or more days the tissue was minced and placed in 50 ml of $CHCl_3$:CH_3OH mixture, 2:1, for 24 hours, doing this 24 hour period at 20° C, the mixture was poured off and fresh mixture was added every 8 hours, i.e., 3 extractions to lipid.

   c. The essentially lipid free was further extracted with 50 ml of anhydrous ethyl-ether for 24 hours, to remove any remaining lipids or water leaving dehydrated-fat-free-tissue (DFFT).

   d. The DFFT was dried in an oven, preset at 50° C, until all odor of ether was gone.

   e. The dry DFFT was suspended in 1.0N PCA and stored at 4° C for a total of 18 hours to remove RNA. The supernatant was removed every 6 hours and the residue re-suspended. Approximately 10 ml of the acid was used with each suspension.

   f. The final residue from (e) was suspended in 10 ml of 1.0N PCA and heated for 30 minutes at 85°C to extract the DNA. This was decanted. A second 10 ml portion of acid was added to the decanted residue and heated again at 85°C for 30 minutes. The

supernatants was added to yield 20 ml of DNA.

g. An aliquot of the DNA extracts were read in the Spectronic 505 at 260 mμ against DNA-PCA standards and an 1.0N PCA blank.

h. An aliquot from the RNA supernatants obtained in (e) were read in the Spectronic 505 at 260 mμ against a PCA blank and RNA-PCA standards.

2. Coenzyme preparations (68)

a. Equal portions of mammary gland tissue (50-250 mg) were placed into 2 glass homogenizers, containing hot 5 ml of 0.1N HCl and 5 ml 0.1NaOH, the containers were placed for 0.5 minute in a hot-water bath, and then homogenized for 1.5 minute.

b. The tissue and fluid were immediately transferred into an ice bath after homogenization until cold.

c. Buffer, 0.5 M tris at pH 8.7, was added to each homogenate and the volume adjusted to 15 ml by the addition of distilled and deionized water.

d. The acid homogenate was then neutralized, cautiously, with 5 ml of 0.1N NaOH (containing oxidized coenzyme) and the alkaline homogenate was neutralized with 5 ml of 0.1N HCl (containing reduced coenzymes).

e. These neutralized homogenates were centrifuged at 20,000 g for 30 minutes at 0° C and filtered to remove fat globules. These preparations were stored at 0° C for assay of the coenzymes.

3. DPN and TPN determinations (27,68)

   a. DPN was reduced in the presence of ethanol (0.5M ethanol in 0.1 M tris at pH10.1) and yeast alcohol dehydrogenase (27). The samples were read in the Spectronic 20 at 340 mμ against standard DPN preparations and recommended enzyme blank.

   b. TPN was reduced in the presence of isocitric dehydrogenase (68), along with DPN, of sodium isocitrate (0.05M), $MgCl_2$ (0.1M) and 0.1 tris at pH 7.4. These were read in the Spectronic 20 at 340 mμ. The total TPN was determined as mentioned in the Results.

4. DPNH and TPNH determinations (27,191)

   a. DPNH was oxidized along with TPNH in the presence of diaphorase (Clostridium kluyveri), tris (0.2M at pH 7.5) and 2,6-sodium dichlorophenolindophenol (27,191). These were read in the Spectronic 505 against standard TPNH-DPNH preparations and the required enzyme blank. The total TPNH was determined as mentioned in the Results.

   b. DPNH was oxidized in the presence of diaphorase (pig heart), tris (0.2M at pH 7.5) and 2,6-sodium dichlorophenol indophenol (27), and were read against appropriate standards and a blank in the Spectronic 505.

5. Histological preparations (107)

   a. A section of mammary tissue was placed on a sheet of filter paper, spread out and allowed to dry for 2-3 minutes.

b. After approximately 3 minutes the paper and tissue were submerged into 10% formalin and left overnight.

c. Upon removal of the paper-tissue preparations from the formalin it was washed in distilled water and stained with alum-carmine for approximately 24 hours (or until the mammary parenchyma was well stained).

d. When properly stained the preparation was washed again in distilled water and dehydrated in graded ethanol (75,80,95, and 100%) for at least one hour--with 2 one-half hour changes.

e. Fat was removed in 2 changes, at least 1 hour each, of toluene.

f. After toluene, the preparation is placed in 2 changes of dioxane to remove the toluene.

g. These fixed preparations upon removal from dioxane were separated, cut at 50μ and embedded.